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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VII. STARCH-LIQUEFYING ACTIVITY, AUTOLYTIC DIASTATIC ACTIVITY AND THEIR CORRELATIONS WITH SACCHARIFYING AND PROTEOLYTIC ACTIVITY¹

By HENRY R. SALLANS² AND J. ANSEL ANDERSON³

Abstract

Determinations made on 144 samples of malt, representing 12 varieties grown at 12 experimental stations in Canada, show that varietal differences exist with respect to starch liquefying activity (max. 768, min. 275 units) and autolytic diastatic activity (max. 958, min. 664 units). Varieties of poor malting quality tend to be low with respect to both properties. The effect of environment is also considerable (liquefying, max. 510, min. 288; autolytic, max. 806, min. 704).

The correlation coefficients among liquefying, autolytic diastatic, saccharifying, and proteolytic activities of malt and total barley saccharifying activity were studied. Significant *inter-varietal* associations exist between each pair of properties, but partial correlation studies suggest that only those between saccharifying activities of barley and malt ($r = 0.90$), and between liquefying and autolytic activities of malt ($r = 0.97$), represent real and close relations. The other associations between pairs of enzymatic activities seem to reflect mainly positive correlations between each activity and total salt-soluble nitrogen in the barleys.

Significant *intra-varietal* associations exist between each pair of enzymatic activities, and between each activity and total barley nitrogen. It appears that environmental factors which tend to increase total nitrogen also tend to increase each enzymatic activity, but these do not increase regularly with respect to each other and are not closely related. Partial correlations independent of total nitrogen suggest that only barley and malt saccharifying activities ($r = 0.67$) and liquefying and autolytic activities of malt ($r = 0.63$) are related within varieties.

It appears that the rate of autolysis in samples of different varieties from the same station is controlled almost entirely by starch liquefying activity, but the latter property is not the limiting factor controlling autolysis in samples of the same variety from different stations. Within varieties some other factor, presumably starch resistance, must play an important part.

Lintner values determined on 144 samples of barley and on the malts made from them were reported in Part II (11) of this series, together with the results of a statistical examination of the relations between these properties. Data on the proteolytic activity of the same malts were given in Part VI (4). Further investigations of the enzymatic activities of these malts, namely,

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² Biochemist, National Research Laboratories, Ottawa.

³ Formerly Biochemist, National Research Laboratories, Ottawa; now Chief Chemist, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.

determinations of starch-liquefying activity and of autolytic diastatic activity, are reported in the present paper.

It is generally agreed that the saccharifying activities of barley (total by the papain method, 11) and of malt measure β -amylase activity. In malt β -amylase doubtless contributes most of the effect, but α -amylase also plays a part in saccharification. The activity of the α -amylase in malt has commonly been measured by methods involving changes in the starch-iodine colour (e.g., 14), but Blom, Bak, and Braae (7) consider that starch-liquefying activity is a better method for estimating this enzyme. They point out, however, that β -amylase has some slight effect on starch-liquefying activity, but conclude that for all practical purposes the liquefying activity can be considered as a measure of α -amylase activity. Considerable doubt has been cast on the validity of the usual starch iodine methods by the recent work of Hanes and Cattle (8). They have shown that the rate of colour change produced by α -amylase is considerably increased in the presence of β -amylase. It, therefore, appears that starch-liquefying methods give a more reliable measure of α -amylase activity than methods based on starch iodine coloration.

It does not yet seem possible to enumerate the factors controlling the autolytic diastatic activity of malt as measured by methods of the Blish-Sandstedt type (5). On *a priori* grounds, it might be assumed that the rate of autolysis is dependent on factors such as starch-liquefying activity, saccharifying activity, and the resistance of the substrate to enzymatic attack. Shellenberger and Bailey (13), working on these assumptions, were unable to demonstrate any close relations among these properties and autolytic diastatic activity.

Blish *et al.* (6, 12) have studied autolytic saccharification in flour and the effect of adding malt extract to the digests. They were concerned mainly with the factors influencing the final degree of saccharification, rather than the rate, and their work bears rather indirectly on the problem under discussion. Further investigation seemed called for and it was mainly for this reason that the work reported in this paper was undertaken.

Since the data were available, a study of the relations among enzymatic activities, *i.e.*, saccharifying (11), proteolytic (4), autolytic diastatic, and starch-liquefying, is given in the present paper.

Materials

The malts used in the study are those used in previous studies in this series (1-4, 10, 11). They represent 12 varieties of barley (listed in Table I), grown at 12 widely separated experimental stations in Canada (listed in Table II). The barley varieties and the methods used in growing the samples were described in detail in Part I (1) of this series and the malting methods and commonly measured properties of the malt, including Lintner values, were reported in Part IV (10).

Methods

Starch-liquefying Activity

The method described by Jozsa and Johnston (9) was used for the determination of starch-liquefying activity. The following modifications were made to facilitate manipulation. Infusions of the malts were made, using 500 ml. of 5% sodium chloride instead of 1 l. of 2.5% solution, and this extract was diluted 1 to 20 with water. Enzymatic digests were made in 250-ml. wide-mouthed Erlenmeyer flasks.

The 100-ml. pipette used to determine viscosity was calibrated by the method of Jozsa and Johnston and it was found that their conversion tables could be used to convert the results to "liquefons" per gram of malt. These authors define a liquefon as "that amount of starch-liquefying enzyme which will convert the standard starch paste at the rate of 25 mg. of dry starch per minute at zero time under the specified conditions."

Autolytic Diastatic Activity

The following modification of the Blish-Sandstedt (5) method was used.

Two 5-gm. aliquots of the finely ground malt were weighed into 100-ml. glass stoppered Florence flasks and subjected to autodigestion at 35° C. Digestion of one aliquot was stopped at the end of one hour in the usual manner and digestion of the second aliquot after two hours. After filtering the digests, 1 ml. of the filtrate was used for a determination of reducing compounds by a modification of the ferricyanide method, which permitted the estimation of larger quantities of sugar. The ferricyanide reagent used was calibrated against a solution of pure maltose, and autolytic activity was expressed as milligrams of maltose produced from 10 gm. of malt during the second hour of digestion.

Typical curves showing the relation between reducing compounds produced and time of digestion for samples of two varieties of barley are shown in Fig. 1. The upper and lower curves represent samples of O.A.C. 21 and Wisconsin 38 grown at the same station. A curvilinear relation exists between 0 and 1 hr., whereas the relation between 1 and 2 hr. is almost linear. Other experiments have shown that if digestion is carried much beyond 2.5 hr. a definite drop in the rate of production of reducing compounds occurs. It seems probable that during the early stages of digestion the fractured starch grains are subjected to ready attack with the result that the initial rate of production of reducing compounds is greatest. When the readily attacked starch has been utilized, the rate of digestion becomes relatively constant until it is retarded by reduced substrate concentration and by the inhibiting effects of hydrolytic products. We have attempted to measure the rate during the period when it is relatively constant.

The dotted curve in Fig. 1 represents the same sample of O.A.C. 21 ground less finely. It will be observed that the two curves for O.A.C. 21 are very similar and it is evident that the method is to some extent independent of the fineness of grinding. This is an important advantage in dealing with samples

grown under widely different environmental conditions and thus differing considerably in their resistance to grinding.

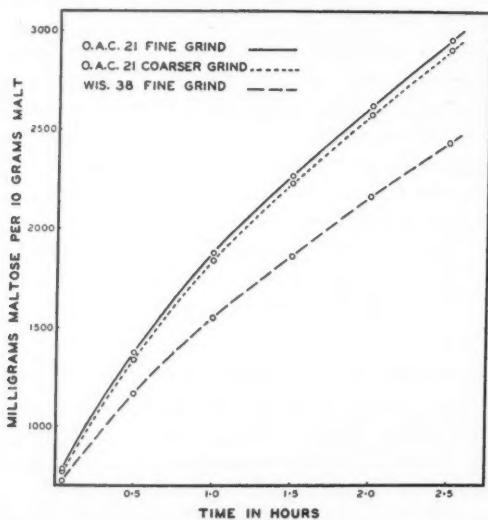


FIG. 1. The relation between reducing substances produced and time of autodigestion. Values represent means of duplicate determinations.

Replication

Duplicate determinations were made on one-third of the samples to provide a check on the precision of the determinations. These replicates were selected at random after imposing the limitation that four samples of each of the 12 varieties and four samples from each of the 12 stations should be selected. The standard deviations of single determinations were: Autolytic activity, mean = 765, S.D. = 20.4 or 2.7% of the mean; starch-liquefying activity, mean = 410, S.D. = 11.0 or 2.7% of the mean.

Mean Differences Between Varieties and Between Stations

The results of the investigation of autolytic activity and starch-liquefying activity are summarized in Table I as means for each variety, over all stations, and in Table II as means for each station, over all varieties. The tables also contain, for purposes of comparison, the corresponding mean values for saccharifying activity which were previously reported in Part II (11).

Owing to the differential effect of environment on varieties, these did not fall in the same order at all stations, nor did the stations fall in the same order with respect to each variety. An analysis of variance was necessary to determine whether differences between varietal means, and between station means, could be considered significant. The results of the analyses are put on record

in Table III, but for the purposes of the following discussion they are summarized (last lines of Table I and II) as necessary differences between means required for a 5% level of significance.

TABLE I

VARIETAL MEANS FOR AUTOLYTIC DIASTATIC ACTIVITY, STARCH LIQUEFYING ACTIVITY AND SACCHARIFYING ACTIVITY OF MALT

Class	Variety	Autolytic diastatic activity, mg. maltose/10 gm.	Starch liquefying activity, liquefons/gm.	Saccharifying activity, "Lintner
Six-rowed, rough-awned	Olli	958	768	153
	O.A.C. 21	785	419	127
	Peatland	779	444	120
	Mensury	777	406	129
	Pontiac	770	412	131
Six-rowed, smooth-awned	Velvet	764	385	124
	Nobarb	678	281	100
	Regal	676	275	85
	Wisconsin 38	664	281	96
Two-rowed, rough-awned	Hannchen	804	418	115
	Victory	787	435	103
	Charlottetown 80	739	392	100
Necessary difference, 5% level		57	54	11

Varietal Differences

The data given in Table I show conclusively that varietal differences exist with respect to each of the three properties measured. Olli gives consistently high values, whereas those for Regal and Wisconsin 38 are consistently low. There is little indication of differences between the various classes of barleys since, although the lowest values are given by three of the smooth-awned varieties, the fourth, Velvet, gives values of about the same order as the rough-awned varieties. In Canada, O.A.C. 21, Mensury, and Olli are considered good malting varieties, whereas Nobarb, Regal, and Wisconsin are considered poor. The data for these varieties thus suggest that good malting varieties are characterized by reasonably high diastatic activities.

Station Differences

The mean values for each station are given in Table II. Comparison of the differences between these with the necessary difference required for a 5% level of significance leaves no room for doubt that environment has a considerable effect on all three properties. It is interesting to note that the maximum spreads between station means, 102 for autolytic activity and 222 for liquefying activity, are considerably lower than the corresponding spreads between varietal means, 294 and 493. Since the stations cover a wide range of environment, from a dry continental climate to a moist maritime one, it

appears that variety has a far greater effect on autolytic diastatic activity and starch-liquefying activity than environment. It is also worth noting that most of the variation between station means, with respect to autolytic activity, is caused by four stations, the first two and last two in the test. Values for the remaining eight stations are closely grouped and do not differ significantly.

TABLE II
STATION MEANS FOR AUTOLYTIC DIASTATIC ACTIVITY, STARCH LIQUEFYING ACTIVITY
AND SACCHARIFYING ACTIVITY OF MALT

Station	Autolytic diastatic activity, mg. maltose/10 gm.	Starch liquefying activity, liquefons/gm.	Saccharifying activity, °Lintner
Fredericton	806	375	85
Ste. Anne de Bellevue	802	393	100
Brandon	788	441	117
Winnipeg	781	428	105
Guelph	778	510	122
Beaverlodge	777	488	150
Ottawa	767	422	133
Gilbert Plains	762	382	133
Nappan	752	288	63
Ste. Anne de la Pocatière	741	424	121
Lacombe	726	418	139
Lethbridge	704	348	116
Necessary difference, 5% level	57	54	11

Analyses of Variance

The variances of the data for each determination were analyzed into portions resulting from (i) average differences between varieties; (ii) average differences between stations; and (iii) remainder. The last portion results not only from variations caused by a true interaction between stations and varieties, but also from variations caused by soil heterogeneity within stations, and by sampling and analytical errors. It, therefore, provides an adequate criterion for testing the significance of differences between station and varietal means.

The mean squares obtained by the analyses of variance are reported in Table III. Since the mean squares resulting from differences in the average performance of individual varieties, and from differences in the average performance of all varieties at different stations, are significantly greater than the corresponding remainders, it is apparent that significant differences exist between varietal means and between station means.

In previous papers of this series, results of analyses of variance of data on 19 properties of the same sets of barleys or malts have been reported. For most of these properties, the variance due to stations proved considerably greater than that due to varieties. It is thus a matter of some interest that the reverse is true with respect to starch-liquefying activity and autolytic diastatic activity. The comparatively greater influence of variety on these properties is thus demonstrated.

TABLE III
ANALYSIS OF VARIANCE FOR STARCH-LIQUEFYING ACTIVITY AND AUTOLYTIC DIASTATIC ACTIVITY

Variance due to	Degrees of freedom	Mean square	
		Starch liquefying activity	Autolytic diastatic activity
Varieties	11	198,909**	7,268**
Stations	11	42,074**	1,098*
Remainder	121	4,337	490

NOTE: In this and later tables ** denotes that the 1%, and * that the 5%, level of significance has been attained.

Correlation Studies

In studying the relations between various barley and malt properties, it is advisable to examine the inter- and intra-varietal correlations separately. These may differ considerably since the former are controlled by genetic factors, whereas the latter are controlled by environmental factors. There is thus little reason for expecting that the inter- and intra-varietal correlations will be similar, and they rarely are. For this reason the two kinds of relations are discussed separately in the following sections.

Inter-varietal Relations

The inter-varietal correlation coefficients between each pair of enzymatic activities are given in Table IV. Since all of these are significant and all but two are highly significant, it is obvious that an inter-varietal association exists between the various activities so that varieties which tend to be high in one enzymatic activity also tend to be high in other enzymatic activities.

It was shown in previous papers of this series that inter-varietal relations existed between barley saccharifying activity, malt saccharifying activity (3), and proteolytic activity (4) on the one hand, and the more soluble barley nitrogen fractions (*e.g.*, non-protein nitrogen, salt-soluble protein nitrogen, and total salt-soluble nitrogen) on the other. Correlations between these

TABLE IV
INTER-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES

Enzymatic activity	Malt			
	Sacchari-fying	Lique-fying	Autolytic diastatic	Proteo-lytic
Barley saccharifying	0.904**	0.750**	0.751**	0.634*
Malt saccharifying	—	.816**	.856**	.662*
Malt liquefying	—	—	.975**	.798**
Malt autolytic diastatic	—	—	—	.807**

activities and total nitrogen, insoluble nitrogen or alcohol-soluble nitrogen were not significant. Similar relations have now been found between starch-liquefying and autolytic diastatic activities and the various nitrogen fractions. The most striking feature of these studies is the existence of significant inter-varietal correlations between all the enzymatic properties and the more soluble nitrogen fractions of the barleys. The correlation coefficients for these activities and total salt-soluble nitrogen are shown in Table V.

TABLE V

INTER-VARIETAL CORRELATION COEFFICIENTS AMONG ENZYMATIC ACTIVITIES (x), TOTAL BARLEY NITROGEN (n) AND SALT-SOLUBLE BARLEY NITROGEN (s)

x = enzymatic activity	Correlation coefficient		
	Simple, r_{xn}	Simple, r_{xs}	Partial, r_{xsn}
Barley saccharifying	0.199	0.739**	0.737**
Malt saccharifying	— .039	.727**	.759**
Malt liquefying	— .103	.727**	.788**
Malt autolytic diastatic	— .136	.750**	.814**
Malt proteolytic	.070	.871**	.882**

To elucidate these inter-varietal relations the regressions of the enzymatic activities on total salt-soluble nitrogen were calculated by stations and tested for homogeneity. This analysis is shown in Table VI. It was found that the regressions did not differ significantly between stations. But, since the individual regressions only account for about one-third of the total variance of the enzymatic activities, it is obvious that total salt-soluble nitrogen is not the most important factor controlling the development of these activities. Similar relations can be shown for the other soluble nitrogen fractions, namely, non-protein and salt-soluble protein nitrogen. It seems probable that these soluble nitrogen fractions reflect some more fundamental property of the barleys that controls the development of the enzymatic activities in the malts.

TABLE VI

TEST OF HOMOGENEITY OF INTER-VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean square				
		Barley saccharifying	Malt saccharifying	Autolytic diastatic	Starch liquefying	Autolytic proteolytic
Differences among station regression coefficients	11	640.85	286.25	9,999.6	14,499	1,267.3
Deviations from individual station regressions	120	727.28	345.01	8,417.4	14,847	1,906.4
Percentage of variance accounted for by individual regressions		36.8	37.5	27.5	34.3	37.0

To determine whether there is any real relation between the pairs of enzymatic activities, apart from that due to a common association with salt-soluble nitrogen, partial correlation coefficients between each pair of activities and salt-soluble nitrogen were calculated. These partial correlation coefficients appear in Table VII. Those between proteolytic and saccharifying activities are of negligible magnitude and it is inferred that the simple correlation coefficients (Table IV) reflect the relations between each of these activities and salt-soluble barley nitrogen.

TABLE VII

INTER-VARIETAL PARTIAL CORRELATION COEFFICIENTS, BETWEEN ENZYMATIC ACTIVITIES, INDEPENDENT OF SALT-SOLUBLE BARLEY NITROGEN

Enzymatic activity	Malt			
	Sacchari- fying	Lique- fying	Autolytic diastatic	Proteo- lytic
Barley saccharifying	0.780**	0.460	0.441	-0.030
Malt saccharifying	—	.610*	.684*	.087
Malt liquefying	—	—	.946**	.399
Malt autolytic diastatic	—	—	—	.437

The following partial correlation coefficients drop below the 5% level of significance: barley saccharifying-liquefying; barley saccharifying-autolytic; proteolytic-liquefying; and proteolytic-autolytic. The available data thus fail to demonstrate that relations exist between these pairs of activities, which are independent of the relations between each of them and salt-soluble nitrogen. Nevertheless, the correlation coefficients are high enough to suggest that loose associations may exist and might be demonstrated by a study of a larger number of varieties.

The relation between barley and malt saccharifying activities is quite close as indicated by the simple correlation coefficient of 0.904. The partial correlation coefficient independent of salt-soluble nitrogen, 0.780, is significant to the 1% level. Since malt saccharifying activity results from the activity of β -amylase already present in the barley, this result was to be expected. When regression of malt saccharifying on barley saccharifying activity was determined by stations, it was found that these regressions did not differ significantly from station to station and would account for 76% of the variance of malt saccharifying activity. Total barley saccharifying activity (papain) is therefore the most important single factor controlling the development of malt saccharifying activity. However, since the removal of those portions of the variance associated with salt-soluble nitrogen affects the relation, it appears that other factors are operating to some extent.

In Part II (11) it was suggested that barley saccharifying activities might be of value in predicting malt saccharifying activities of plant breeders' samples. It was also stated that the lack of complete correspondence between

the two activities might be due either to the saccharifying activity of α -amylase or to differences in the responses of varieties to the particular malting conditions used in the investigation. The latter seems more probable, since the multiple correlation coefficient between malt saccharifying activity and barley saccharifying and starch-liquefying activities, 0.928, is not significantly higher than the simple coefficient, 0.904, but the multiple correlation coefficient between malt saccharifying activity and barley saccharifying activity and index of nitrogen modification, 0.952, is significantly higher than the simple coefficient.

A significant degree of association persists between malt saccharifying and liquefying activities, independent of salt-soluble nitrogen. It thus appears that there may be a direct association between these two activities.

The inter-varietal relation between starch-liquefying and autolytic diastatic activities is very close ($r = 0.975$; partial, independent of salt-soluble nitrogen, $= 0.946$). Individual regression equations of autolytic on liquefying activities account for 81.5% of the variance due to autolytic activity. It therefore appears that the liquefying activity of α -amylase is a factor of primary importance governing the rate of autolysis of malts made from barleys grown at the same station.

The relative importance of the saccharifying and liquefying activities in controlling the rate of autolysis in samples of different varieties can be demonstrated by the following partial correlation coefficients.

Autolytic \times liquefying, independent of saccharifying, 0.934**.

Autolytic \times saccharifying, independent of liquefying, 0.466.

Since the first of these is highly significant, whereas the second is not significant, it is clear that liquefying activity is a major, and saccharifying activity a minor, factor governing the rate of autolysis. This is also shown by the fact that the multiple correlation coefficient for autolytic activity and liquefying and saccharifying activities, 0.981, is not significantly higher than the simple coefficient between autolytic and liquefying activities.

Intra-varietal Relations

It has been shown throughout these studies that within varieties almost all properties are correlated with total barley nitrogen. In these circumstances simple intra-varietal correlation coefficients are frequently misleading, since they tend to reflect correlations between nitrogen and the variables under consideration, rather than the true relations between the variables. For this reason the intra-varietal relations discussed in this section are represented in Table VIII by both simple correlation coefficients and partial correlation coefficients independent of total nitrogen.

In considering the simple correlation coefficients given in the upper part of Table VIII, it is simplest to note first that autolytic diastatic activity is not significantly correlated with any of the other properties. It appears that within varieties autolytic activity is controlled by a number of factors, which vary independently with change in environment. Among these factors lique-

fyng and saccharifying activity are doubtless numbered, but the roles they play are not of sufficient importance to produce significant simple correlation coefficients.

TABLE VIII

INTRA-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES AND TOTAL BARLEY NITROGEN, AND PARTIAL CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES INDEPENDENT OF TOTAL BARLEY NITROGEN

Enzymatic activity	Total barley nitrogen	Malt			
		Sacchari- fying	Lique- fying	Autolytic diastatic	Proteo- lytic
Simple correlation coefficients					
Barley saccharifying	0.976**	0.978**	0.589*	−0.302	0.841**
Malt saccharifying	.962**	—	.690*	— .234	.879**
Malt liquefying	.632*	—	—	.300	.728**
Malt autolytic diastatic	— .269	—	—	—	.026
Malt proteolytic	.854**	—	—	—	—
Partial correlation coefficients, independent of total nitrogen					
Barley saccharifying	—	.669*	— .162	— .189	.070
Malt saccharifying	—	—	.386	.095	.406
Malt liquefying	—	—	—	.630*	.467
Malt autolytic diastatic	—	—	—	—	.510

All other activities are correlated with total nitrogen and among themselves. It thus appears that a change in environment that increases total barley nitrogen also tends to increase each of the enzymatic activities under consideration. Among the inter-station correlation coefficients only that between barley and malt saccharifying activities is particularly high. It thus appears that these two activities are quite closely related within varieties as well as between varieties.

Among the partial correlations independent of total nitrogen, only two are significant. One of these represents the relation between barley and malt saccharifying activities.

The other significant partial correlation coefficient is that between autolytic activity and liquefying activity. It is apparent that some relation exists between these two activities within varieties (as well as between varieties) but that this relation is masked in the simple correlation by the complicating effect of correlations with total nitrogen. It is possible that within varieties total nitrogen is correlated with some factor that has a considerable influence on autolytic activity, for instance, substrate resistance. When the effects of differences in total nitrogen are removed by calculating the partial correlation coefficient, some of the effects of differences in substrate resistance are probably removed also. Under these conditions it then becomes possible to demonstrate the relation between starch-liquefying activity and rate of autolysis, *i.e.*, the partial correlation coefficient is found to be significant.

In general, there is some degree of similarity between the inter- and intra-varietal correlations. In both instances a close relation exists between barley and malt saccharifying activities, starch-liquefying activity is shown to be an important enzymatic factor governing rate of autolysis, and rather loose associations exist between the other pairs of activities.

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PHYSIOLOGICAL ACTIVITY OF A SERIES OF INDOLYL ACIDS¹

BY N. H. GRACE²

Abstract

The physiological activity of a series of indolyl acids, from the acetic to the valeric, including 5-methyl-indolylpropionic, has been determined by the rooting responses of *Lonicera tartarica* cuttings treated with solutions of each. Indolyl-butyric acid was the most active chemical, affecting the number and length of roots per rooted cutting, the mean root length, the green weight of leaves, and the fresh root weights. Indolylacetic acid had significant effects on the number and length of roots per rooted cutting. Slight activity was shown by indolylpropionic acid, but neither indolylvaleric acid nor 5-methyl-indolylpropionic showed any significant treatment effects. None of the acids affected the number of cuttings rooted.

A recent communication gives the results of experiments in which plant cuttings were treated with a series of naphthyl acids from 1-naphthyl-acetic to ϵ -(1-naphthyl)-hexoic (2). It was demonstrated that physiological activity was shown by all members of the series tested, and the greater activity of the acids with an even number of carbon atoms in the side chain was the most striking feature of the results. It was considered of interest to carry out a similar experiment with a series of indolyl acids. In consequence, *Lonicera tartarica* L. cuttings, as used previously, were treated with indolyl acids from indolyl-3-acetic to α -(3-indolyl)-valeric acid, and with β -3(5-methyl-indolyl)-propionic acid.

Experimental

The details of statistical arrangement and treatment were identical with those previously described, excepting that four, not five, replicates of 10 cuttings were used in this experiment (2). As with the naphthyl acids, solutions were prepared in 100 p.p.m. of K_2HPO_4 , and this concentration of phosphate was used on the controls and with each chemical at all three concentrations, namely: 10, 50, and 100 p.p.m.

Dormant cuttings* were approximately 12 in. long, and were treated in groups of 40 (the four replicates) in 150 cc. of solution for 24 hr.; 720 cuttings were required for the experiment. They were rinsed and immediately planted in random order in brown sand in a propagation frame equipped with bottom heat cables. Sand temperature was maintained at 72° F., while that of the room approximated 65° F. The present experiment received more light than the former, as the days were longer during the present experiment. Apart from light effects the series of treatments of *Lonicera tartarica* with naphthyl and indolyl acids were carried out under closely similar conditions. The

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. No. 854.

² Biochemist, National Research Laboratories, Ottawa.

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cuttings were planted March 9 and removed for counts and measurements April 19, 1939.

In addition to the counts and measurements made on *Lonicera* treated with naphthyl acids (number of cuttings rooted, number and length of roots per rooted cutting, and the mean root length), weight of leaves and fresh root weights were determined. All the data thus secured were subjected to analyses of variance.

Results

The number of cuttings rooted in this experiment achieved a rather high general level; over all, 65% of those planted formed roots, as contrasted with about 35% rooting for untreated controls in previous studies with this plant (3, 4). Apparently this general stimulation was due to a uniform treatment of nutrient phosphate, K_2HPO_4 ; nutrient treatments of *Lonicera* cuttings have been shown to stimulate rooting (5). The response of this collection of cuttings to nutrient treatment may have masked the effects of the hormone on the number rooted.

It will be observed from Table I that there were significant differences due to the use of chemicals in respect of the remaining five characters. No significant interaction could be demonstrated; i.e., there was no differential response at different dosages to the same chemical.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF *Lonicera tartarica* TO A SERIES OF INDOLYL ACIDS

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings rooted (transformed data)*	Length of roots per cutting rooted	Mean root length, mm.	Number of roots per cutting rooted	Green weight of leaves	Fresh weight of roots
Replicates	3	479.0	17499	120.3	15.74	18.18	1.064
Dosages	2	6.8	51050	236.7	34.07	2.24	0.027
Error (a)	6	671.2	45636	121.7	27.40	18.40	2.557
Chemicals	5	173.7	168639***	149.7**	411.47***	14.42*	3.108**
Chemicals \times dosages	10	136.0	22814	65.8	23.76	7.64	1.418
Error (b)	45	190.9	18182	42.3	25.93	4.25	0.787

S Data subjected to inverse sine transformation (1).

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

Table II shows the effect of chemicals, as estimated from the differences between treated and control means. Indolylbutyric acid increased the length and number of roots per rooted cutting and the fresh root weight, but decreased the mean root length and the green weight of leaves. Indolylacetic acid increased the length and number of roots per rooted cutting. Indolyl-

propionic acid decreased the mean root length and the green weight of leaves. Neither 5-methyl-indolylpropionic nor indolylvaleric acids had any significant effect on the responses studied.

TABLE II

RESPONSE OF *Lonicera tartarica* TO A SERIES OF INDOLYL ACIDS

Treatment	Number of cuttings rooted (transformed data) ^S	Length of roots per cutting rooted, mm.	Mean root length, mm.	Number of roots per rooted cutting	Green weight of leaves, gm.	Fresh weight of roots, gm.
Control	55.7	241	30	8	8.6	1.23
Indolylacetic	55.2	377*	30	12*	7.4	1.58
Indolylpropionic	49.3	260	23*	11	6.4*	0.84
5-Methyl-indolylpropionic	51.4	234	25	10	7.4	1.23
Indolylbutyric	56.9	544*	24*	24*	5.6*	2.21*
Indolylvaleric	59.8	321	31	11	8.0	1.95
Necessary difference for 5% of significance		111	5.3	4	1.7	0.73

^S Data subjected to inverse sine transformation (1).

* Exceeds 5% level of significance.

Indolylbutyric acid was the most consistently effective of the chemicals employed; and the general effect of chemicals was to increase the number while decreasing the individual length of roots, and to increase total root weight while reducing the weight of tops.

The results would appear to indicate that members of the indolyl series with an even number of carbon atoms in the side chain have greater physiological activity than those with an odd number, a conclusion in essential agreement with the variation of activity in the naphthyl series. However, while there was little difference in the activity of naphthylbutyric and -acetic acids, in the indolyl series the butyric acid was markedly more active than the acetic homologue.

Acknowledgment

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VEGETATIVE PROPAGATION OF CONIFERS

III. EFFECT OF MONTH OF COLLECTION ON THE ROOTING
OF DORMANT NORWAY SPRUCE CUTTINGS¹BY N. H. GRACE²

Abstract

In a comparison of the rooting responses of cuttings of Norway spruce from upper branches, in which one lot was stored under snow from November until early April and the other collected in late March, there were statistically significant differences. After 12 weeks 38.9% of the November, and 20.7% of the March, cuttings were rooted or calloused. At the end of the propagation period 45.2% of the November, and 66.6% of the March, cuttings were dead. Indolylacetic acid dust treatment did not have a significant effect on the number of cuttings rooted or calloused; it did, however, increase the number that were dead at the end of the experiment.

Among the factors of possible importance in the vegetative propagation of conifers are the effects of period of collection and storage on the rooting of dormant cuttings. Deuber and Farrar have shown recently that there is a marked change in the rooting of cuttings of *Picea excelsa* Link. over the period of October to January (2). The present communication describes a preliminary experiment in which the effect of time of collection on the rooting of Norway spruce cuttings was studied.

Experimental

Branches of Norway spruce were collected in mid-November 1938 and buried under snow until early April (3, 4). These were compared for rooting ability with a collection of branches made in March 1939.* Both collections were from the upper part of trees from a plantation approximately 18 years of age, on the Petawawa Forest Experiment Station, Chalk River, Ontario. Cuttings ranged from two to four inches in length and had a heel of old wood, and were divided into groups of 13 cuttings, each representative of the various lengths. Prior to planting, four replicates of 13 cuttings of each collection were treated with talc only, with 100, and with 1000 p.p.m. (parts per million) of indolylacetic acid in talc. There was also an untreated control.

The cuttings were planted April 5, 1939, in brown sand in a propagation frame equipped with bottom heat cables. The propagation frame was provided with a factory cotton screen, which reduced light intensity and assisted in maintaining the humidity. The random order of planting resulted

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² Biochemist, National Research Laboratories, Ottawa.

* The staff of the Petawawa Forest Experiment Station, Chalk River, Ontario, kindly made the March collection.

in one level of precision for all the comparisons. Sand temperature was maintained at 72° F., while the room temperature ranged between 65 and 70 F° for the first few weeks of the propagation period. However, during the last eight weeks the room temperature frequently went up to from 80 to 95 °F. during the day.

The cuttings were removed 12 weeks after planting and the number rooted, calloused, and dead, and the number and length of roots were determined. Poor rooting of the March collection rendered it impossible to make a statistical analysis of the data for the number and length of roots per rooted cutting. This led to combination of the number of cuttings calloused with those rooted for statistical analysis, based on inverse sine transformations (1).

Results

Data for the number of cuttings rooted or calloused and for the number dead were subjected to analyses of variance, and the results are presented in Table I. The number of cuttings rooted or calloused was affected by the

TABLE I

ANALYSES OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS COLLECTED IN NOVEMBER AND MARCH AND TREATED WITH DUSTS CONTAINING INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square	
		Number of cuttings calloused or rooted	Number of cuttings dead
Replicates	3	136.24	49.82
Indolylacetic acid dosage	3	260.59	238.32*
Month of collection	1	1309.44***	1352.00***
Interaction Indolylacetic acid treatment × month of collection	3	102.37	296.37*
Error	21	86.93	66.62

* Exceeds mean square error, 5% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

month of collection, but neither indolylacetic acid treatment nor the interaction between indolylacetic acid treatment and month of collection had a significant effect. The number of cuttings dead showed that the month of collection was significant to the 0.1% level, and indolylacetic acid treatment and the interaction between treatment and month of collection also were significant, but only to the 5% level.

In Table II are given data for the effects of the month of collection on the number of cuttings rooted or calloused and the number dead. The November

TABLE II

THE EFFECT OF MONTH OF COLLECTION ON THE NUMBER OF DORMANT NORWAY SPRUCE CUTTINGS ROOTED OR CALLOUSED

Month of collection of cuttings	Cuttings			
	Rooted or calloused		Dead	
	Transformed data	Per cent	Transformed data	Per cent
November	38.2	38.9	42.1	45.2
March	24.4	20.7	55.1	66.6
Necessary difference, 5% level	6.9		6.1	

collection gave significantly more rooted or calloused cuttings, and fewer dead cuttings than the March collection. There were 19.7% of the November cuttings rooted, while only 2.9% of the March collection rooted.

The effects of indolylacetic acid treatment and the month of collection on the number of cuttings that died are shown in Table III. Both 100 and 1000 p.p.m. treatments increased mortality of November cuttings over the untreated control; the untreated and talc groups did not differ. The response of March cuttings to treatment was somewhat different in that 1000 p.p.m. indolylacetic acid caused significantly greater mortality than was shown by the untreated or 100 p.p.m. groups. The interaction effect was due to this difference in response of cuttings of the two collections to 100 p.p.m. indolylacetic acid treatment; in March, this treatment, while not differing significantly from the untreated control, suggested a decrease in mortality. The treatment means indicated that 1000 p.p.m. indolylacetic acid in talc increased mortality over the untreated group; values for the other two treatments were intermediate but did not differ significantly from the latter.

TABLE III

EFFECTS OF MONTH OF COLLECTION AND INDOLYLACETIC DUST TREATMENT ON THE NUMBER OF NORWAY SPRUCE CUTTINGS DEAD

Month of collection of cuttings	Untreated	Indolylacetic acid in talc, p.p.m.			Necessary difference, 5% level
		0	100	1000	
Transformed data					
November	34.7	36.6	49.6	47.3	12.0
March	50.7	60.1	45.1	64.5	
Treatment means	42.7	48.4	47.4	55.9	8.5

The results are in general agreement with those of Deuber and Farrar, and suggest that there is a marked change in rooting ability during the dormancy period (2). It would appear that branches stored under snow, following collection in November, do not undergo physiological changes to the same extent as if left on the tree throughout the winter. It may not be assumed that such storage completely stops all physiological change, since November cuttings, the collection used in this experiment, responded favourably to 1000 p.p.m. indolylacetic acid in talc when treated in December (4). However, close comparisons cannot be drawn between the former and present experiment, since in the one the cuttings were plain and in the other they had a heel of old wood. Further, propagation conditions varied markedly owing to summer temperatures.

It would appear that the rooting of dormant Norway spruce cuttings depends to a greater extent on the period of collection than on treatment with root growth stimulating chemicals.

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THE EFFECT OF LEAF RUST ON THE YIELD AND QUALITY OF THATCHER AND RENOWN WHEAT IN 1938¹

BY B. PETURSON² AND MARGARET NEWTON³

Abstract

A study was made at Winnipeg in 1938 to determine the effect of leaf rust on the yield and quality of Thatcher and Renown wheat. In one experiment, Thatcher and Renown were sown late in 1/400-acre plots; in another, Thatcher only was used and was sown early in rod-row plots. Half the plots of each variety were kept as free from leaf rust as possible by frequent applications of sulphur dust, but the remaining half became heavily infected. In the 1/400-acre plots, leaf rust reduced the yield of Thatcher and Renown by 51.17 and 29.61%, respectively; in the rod-row plots of Thatcher, it reduced the yield by 37.02%. The decrease in yield was due more to reduction in kernel weight than to reduction in number of kernels per head. All the non-dusted plots ripened approximately three days earlier than the dusted, and the grain from them graded one grade lower than that from the corresponding dusted plots. In both varieties, the protein content was diminished while the carotene content was increased.

Stem rust (*Puccinia graminis Tritici* Erikss. & Henn.) and leaf rust (*Puccinia triticina* Erikss.) have caused a great deal of damage to the wheat crop in the Prairie Provinces of Canada. Generally speaking, when stem rust has been severe on wheat, leaf rust has been somewhat abundant on it also. For this reason, in studies (2) already made in the Prairie Provinces on the effect of stem rust and leaf rust on the yield and quality of wheat, an exact allocation of the damage caused by either of these rusts could not be made. Undoubtedly a great proportion of the injury has been attributable to stem rust, as Marquis, the common wheat formerly chiefly grown, was only moderately susceptible, and Mindum, the only durum variety widely grown, has been rather resistant to leaf rust.

That leaf rust of wheat is capable of doing extensive damage has already been shown. Caldwell *et al.* (1), Hayes *et al.* (3), Johnston (4), Johnston and Miller (5), Mains (6), Melchers (7), and Waldron (9) in the United States, and Phipps (8) in Australia have shown that leaf rust very materially reduced the yield of wheat.

With the introduction into the Prairie Provinces of new varieties of wheat resistant to stem rust, some of which are quite susceptible to leaf rust, an opportunity was afforded for ascertaining to what extent the new wheats might be injured by leaf rust. Experiments were planned, therefore, to determine the effect of leaf rust on the yield and quality of two of the new varieties resistant to stem rust, namely, Thatcher and Renown.

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Contribution No. 591, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

² Assistant Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

³ Senior Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

Materials and Methods

Two different experiments were carried out. In the first, the varieties Thatcher and Renown were grown on summer-fallowed land in 1/400-acre plots. There were 12 plots of each variety. Half the plots of each variety were kept as free from rust as possible by dusting them three times a week, from June 23 to August 4, with sulphur (Kolodust) at the rate of 30 lb. per acre per dusting. The remaining plots were not dusted. The plots were not artificially inoculated with leaf rust but were sown late (May 23) in order to permit the rust to develop as fully as possible on them. Both varieties were sown at the rate of $1\frac{1}{2}$ bu. per acre.

In the second experiment the variety Thatcher only was used. It was sown early (May 11) in rod-row plots. Each plot consisted of three rod-rows spaced one foot apart and sown on summer-fallowed land at the rate of 500 seeds per row. There were in all 25 pairs of plots. During the third week in June, one plot of each pair was artificially inoculated with leaf rust. The non-inoculated plots of each pair were dusted at the same rate and time as those in the first experiment. The centre rows only of each plot were harvested.

In both experiments, leaf rust percentages were estimated while the leaves were still green, stem rust percentages, just before the plants ripened. The estimate of rust percentages was based on the scale of stem-rust percentages adopted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. For each individual plot in both experiments, the yield, number of kernels per head, bushel weight, and 1,000-kernel weight were taken, and, through the courtesy of the Western Grain Inspection Division, Winnipeg, commercial grades were obtained on the bulked dusted and non-dusted samples of each variety. The bulked samples of grain from the dusted and the non-dusted plots of both experiments were each tested for baking quality.

Experimental Results

Severity of Infection in Plots

A severe outbreak of leaf rust of wheat occurred in Manitoba in 1938. All the non-dusted plots of Thatcher, both in the artificially and in the naturally infected plots, became heavily rusted; those of Renown were less heavily rusted. In the 1/400-acre plots of Thatcher, the infection averaged 68 and 32% in the non-dusted and dusted plots, respectively; in the Renown plots, 33% in the non-dusted plots and 13% in the dusted plots. In the rod-row plots of Thatcher, leaf-rust infection averaged 77% in the non-dusted plots, and 32% in the dusted plots. Only very slight traces of stem rust developed in the Thatcher and Renown plots. That is to say, a few pustules occurred at the nodes of a small percentage of the plants. These pustules were not sufficiently numerous to have any appreciable effect on the yield of the two varieties, either in the dusted or non-dusted plots.

Effect of Leaf Rust on Yield, Weight per Measured Bushel, and Grade of Thatcher and Renown

The grade, yield, and weight per measured bushel of the dusted and non-dusted 1/400-acre plots of Thatcher and Renown, and the rod-row plots of Thatcher are given in Table I. From these results it is evident that leaf rust of wheat reduced the yield of the 1/400-acre plots of Thatcher and Renown (sown late), and of the rod-row plots of Thatcher (sown early). In the 1/400-acre plots, the non-dusted Renown yielded 29.61% less than the dusted, and the non-dusted Thatcher, 51.17% less than the dusted. In the rod-row plots, the non-dusted Thatcher yielded 37.02% less than the dusted. Thatcher was, therefore, more seriously affected than Renown and when sown late was damaged appreciably more than when sown early.

TABLE I

THE EFFECT OF LEAF RUST ON THE YIELD, BUSHEL WEIGHT, AND GRADE OF THATCHER AND RENOWN GROWN IN FIELD PLOTS AT WINNIPEG IN 1938

Type of plot	Variety	Treatment	Time of sowing	Leaf rust, %	Average yield per acre, bu.	Decrease in yield per acre due to leaf rust, %	Average weight per bushel, lb.	Grade (Northern)
1/400-acre	Thatcher	Dusted	Late-sown**	31	23.86	51.17	62.10	No. 2
		Non-dusted	Late-sown**	68	11.65		56.80	No. 3
	Renown	Dusted	Late-sown**	13	24.75	29.61	64.10	No. 1
		Non-dusted	Late-sown**	33	17.42		62.20	No. 2
Rod-row	Thatcher	Dusted	Early-sown*	32	31.56	37.02	64.60	No. 2
		Non-dusted	Early-sown*	77	19.86		61.00	No. 3

*Sown May 11.

**Sown May 23.

Leaf rust also influenced the length of the ripening period. Although both the dusted and non-dusted plants of early-sown Thatcher came into head on the same day, the non-dusted plants ripened three days earlier than the dusted plants.

The weight per measured bushel and grade of both these varieties were also adversely affected by leaf rust. The grain from the non-dusted plots of early-sown Thatcher, late-sown Thatcher, and Renown plots weighed 3.6, 5.3, and 1.9 lb. per bu. less, respectively, than the grain from the corresponding dusted plots. In each case the grain of the non-dusted plots graded one grade lower than that of the corresponding dusted plots.

The Effect of Leaf Rust on Kernel Weight and Number of Kernels per Head

Reduction in yield by leaf rust of wheat may be brought about in different ways. Mains (6) found that, in Illinois, reduction was due to two main

causes: (a) the tip and basal spikelets of the head, as well as other spikelets in the remaining portion of the head, often failed to set seed with the result that the number of kernels per head was reduced; and (b) the individual kernels did not attain their normal size and plumpness. In Kansas, Johnston and Miller (5) found that reduction in grain yield was due, primarily, to the production of fewer kernels by rusted plants and, secondarily, to reduced kernel weight. Similarly, Caldwell *et al.* (1) in Illinois, and Johnston (4) in Kansas, found that reduction in yield was due mainly to a reduction in the number of kernels per head. Waldron (9), on the other hand, found that, under field conditions in North Dakota, the reduction in yield from leaf rust was due mainly to a reduction in the weight of the kernels.

These discrepancies in results may be explained, in part at least, by the fact that the degree of kernel reduction may depend on the stage of development of the plants at the time of the onset of the rust. Mains (6) pointed out that when infection occurred relatively early, reduction in yield was due chiefly to a reduction in the number of kernels, but when infection occurred late, to a reduction in kernel weight and, to a much less extent, to a reduction in number of kernels. The explanation of this change appears to be that if severe rust infection occurs sometime before fertilization takes place, the number of seeds formed may be materially reduced, whereas, if the infection takes place after fertilization occurs, the number of seeds produced may not be greatly reduced.

An attempt was made, therefore, to determine at Winnipeg if the early-sown Thatcher, infected by leaf rust at a late stage, would show a smaller reduction in number of kernels than the late-sown Thatcher, infected at an early stage; and if the reduction in yield in the early-sown Thatcher would be due chiefly to a reduction in kernel weight, while that of the late-sown Thatcher and Renown would be due chiefly to a reduction in number of kernels. The loss in kernel weight was determined by obtaining the weights of 1,000-kernel lots, taken at random from the threshed grain samples of the individual dusted and non-dusted plots, both of the early and late-sown plots. The number of kernels produced per head was determined by ascertaining the average number of kernels per head of plants selected at random from the outside rows of the dusted and non-dusted plots. For this purpose, 20 heads were taken from each plot.

The results presented in Table II show that in the early-sown Thatcher (infected at heading time) there was a reduction in number of kernels of 7.00%, while in the late-sown Thatcher (infected two weeks before heading) there was a reduction of 17.41%. However, as will be seen in Table II, the reduction in yield in the early-sown Thatcher, the late-sown Thatcher, and Renown, sown at the same time as late-sown Thatcher, were due more to a reduction in kernel weight than to reduction in number of kernels. For example, the reduction in kernel weight of the grain in the non-dusted plots of the early-sown Thatcher, the late-sown Thatcher, and Renown amounted to 26.47, 27.08, and 16.16%, respectively, whereas the reduction in number of kernels

per head in the same plots amounted to 7.00% in the early-sown Thatcher, 17.41% in the late-sown Thatcher, and 5.74% in Renown. That is to say, in the early-sown Thatcher, 77.8% of the loss in yield in the non-dusted plots was due to reduction in kernel weight and the remaining 22.2% to reduction in number of kernels per head. In the late-sown Thatcher and in Renown, reduction in kernel weight amounted to 56.2 and 66.6%, respectively, and reduction in number of kernels to 43.8% in Thatcher and 33.4% in Renown.

TABLE II

THE EFFECT OF LEAF RUST ON THE WEIGHT PER 1,000 KERNELS AND NUMBER OF KERNELS PER HEAD OF THATCHER AND RENOWN GROWN IN FIELD PLOTS AT WINNIPEG IN 1938

Type of plot	Variety	Treatment	Time of sowing	Average weight per 1,000 kernels, gm.	Decrease in weight per 1,000 kernels due to leaf rust, %	Average number of kernels per head	Decrease in number of kernels per head due to leaf rust, %
1/400 acre	Thatcher	Dusted	Late-sown**	22.63	—	22.40	17.41
		Non-dusted	Late-sown**	16.50	27.08	18.50	
	Renown	Dusted	Late-sown**	30.01	—	17.75	5.74
		Non-dusted	Late-sown**	26.36	16.16	16.73	
Rod-row	Thatcher	Dusted	Early-sown*	27.81	—	22.36	7.00
		Non-dusted	Early-sown*	20.45	26.47	20.79	

*Sown May 11.

**Sown May 23.

In these trials, the epidemic of leaf rust in the late-sown plots did not reach a maximum until the plants were almost in head, and, in the early-sown plots, until after the plants were in head. Had the attack of leaf rust become severe at a very early stage in the life of the plants, the ratio of loss in number of kernels to loss in weight of kernels would probably have been different. In view of the fact that, in the Prairie Provinces, leaf rust seldom becomes well established until the wheat plants are almost in head, it seems probable that, as a rule, the reduction in yield by leaf rust is due more to reduced size than to reduced number of kernels.

All the data pertaining to yield, bushel weight, and 1,000-kernel weight, but not those pertaining to baking and milling tests, were subjected to statistical analysis, and the differences were found to be significant.

Effect of Leaf Rust on the Milling and Baking Qualities of Thatcher and Renown

Grain samples from the dusted and non-dusted plots of Thatcher and Renown were submitted for quality tests to the Grain Research Laboratory of the Board of Grain Commissioners, Winnipeg, and to the Cereal Division, Experimental Farms Service, Ottawa. As the results of these two tests

TABLE III
MILLING AND BAKING TESTS CONDUCTED BY THE CEREAL DIVISION, EXPERIMENTAL FARMS BRANCH, OTTAWA
(MALT-PHOSPHATE-BROMATE METHOD)

Type of plot	Sample	Treatment	Wheat protein, %	Flour yield, %	Flour colour (carotene), p.p.m.	Absorption, %	Dough character	Loaf volume, cc.	Loaf ¹ form	Crust ¹ colour	Crumb ¹ texture	Crumb ¹ colour
1/400 acre	Late-sown Thatcher	Dusted	14.5	74.0	2.48	59	Strong	847	5	5	8.6	7.3
		Non-dusted	14.0	71.7	3.00	60	Strong	877	5	5	8.0	5.6
	Late-sown Renown	Dusted	15.5	73.4	2.15	60	Strong	921	5	5	8.3	7.6
		Non-dusted	15.3	73.0	2.53	59	Strong	914	5	5	8.3	7.3
Rod-row	Early-sown Thatcher	Dusted	15.6	72.0	2.25	61	Strong	818	5	5	8.4	7.4
		Non-dusted	14.6	72.6	2.97	61	Strong	854	5	5	8.0	5.6

¹ Maximum scores: Loaf form 5; Crust colour 5; Crumb texture 10; Crumb colour 10.

were in agreement, it seems necessary to include only one set of data. That furnished by the Cereal Division is presented in Table III. The results show that the grain from the dusted plots was higher in protein content, but lower in carotene content, than that from the non-dusted plots. The crumb colour of the bread baked from wheat from the dusted plots, particularly the Thatcher plots, was superior to the crumb colour of that baked from the wheat from the non-dusted plots. With Renown wheat, the loaf volume was greater in the samples from the dusted plots than from the non-dusted plots. The reverse, however, was true of Thatcher. In all the other milling and baking characteristics, the grain from the dusted and non-dusted plots gave very similar results.

Discussion

A true measure of the damage caused by leaf rust of wheat was not obtained in these experiments as leaf rust was not completely controlled in the dusted plots. If leaf rust had been suppressed completely in these plots, the differences in yield and quality of the grain from the dusted and non-dusted plots would undoubtedly have been greater. These experiments, however, clearly show that, under severe leaf-rust infection such as prevailed throughout most of Manitoba in 1938, leaf rust materially reduced the grade, yield, and quality of Thatcher and Renown, and that Thatcher, the more susceptible of these two varieties, suffered greater damage than Renown. In all these tests the differences in grade, yield, and quality of grain from corresponding dusted and non-dusted plots can be attributed to the difference in amount of leaf rust on the plants and not to any direct beneficial effect of sulphur to the plants. Experiments conducted by Greaney (2) at Winnipeg have shown that, in the absence of rust and other leaf and stem diseases, the dusting of wheat varieties with sulphur during the growing period has no appreciable effect on yield.

In the present experiment, the rusted plants ripened three days earlier than those kept free from rust by sulphur dust. This shortening of the ripening period can probably be attributed to the effect of the fungus on the wheat plants, and also to a lack of moisture in the soil, for, at Winnipeg, during the growing season of 1938, the precipitation was abnormally low. Weiss (11) studied the water requirement of Marquis wheat when infected with stem rust and leaf rust, and found that the rusted plants had a higher water requirement than the non-rusted ones, although the differences were significant only in the case of stem-rust infection. Weaver (10) compared the rate of transpiration in rusted and non-rusted wheat, rye, barley, and oats, and found that the transpiration rate was consistently higher in the rusted plants. Johnston and Miller (5) found that, in the greenhouse, when heavy leaf-rust infection occurred early in the growth of the plants, the water requirement of a susceptible wheat variety was more than doubled. It would seem, therefore, that, in the field experiments at Winnipeg, the rusted plants did not have a large enough water supply to meet their increased needs, and, consequently, matured early. In the greenhouse experiments by Johnston

and Miller (5), the rusted plants were abundantly supplied with water and ripened later than those free from rust. Some observations made by the writers at different times seem to indicate that when wheat plants are infected with leaf rust, but well supplied with water, the maturity appears to be delayed. It is just possible that, had the precipitation at Winnipeg in 1938 been excessive, leaf rust might have had the opposite effect, namely, to delay the ripening of the rusted plants.

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EFFECTS OF TEMPERATURE AND SUNLIGHT ON THE RATE OF ELONGATION OF STEMS OF MAIZE AND GLADIOLUS¹

BY A. G. MCCALLA,² J. R. WEIR³, AND K. W. NEATBY⁴

Abstract

Measurements of the height of the main stems of maize and gladiolus plants were made at four-hour intervals starting at 4 a.m. The increases in elongation were analyzed statistically, together with mean temperature, hours of sunlight, and mean relative humidity.

Partial correlation coefficients showed that there was a highly significant positive association between the rate of stem elongation (growth) and temperature, regardless of the time of day, the variations in temperature accounting for from 40 to 70% of the variability in growth rates. There was likewise a significant negative correlation between growth and sunlight, but sunlight was apparently effective only during the midday periods (8 a.m. to 4 p.m.). The depressing effect of sunlight on the growth of gladiolus was approximately four times as great as on maize. This depressing effect on maize was entirely removed by shading the plants with light white cotton.

Variations in relative humidity were only slightly associated with growth rates. No significant effect was observed for any of the periods. It seems possible that these factors might be more important under conditions of deficiency in soil moisture.

The approximate minimum temperature at which growth took place was 40° F.

Introduction

Many years ago it was pointed out by Reed (22) that although much study had been given to the effects of external factors on total growth, little had been given to the effects of the same factors on rate of growth. Since that time there has been a considerable amount of work carried out in an effort to determine the effects of external factors on rate of growth, but no general agreement as to the relative importance of the different factors has been reached. The factors most generally considered are temperature, sunlight, relative humidity, and rainfall. The last mentioned is related to the internal condition of the plant and also plays a part in determining relative humidity. If soil moisture is not limiting, the latter effect is probably the more important.

Probably more attention has been given to the effects of temperature than to the effects of any other factor, although many investigators have considered sunlight as an important factor in determining rate of growth. With respect to the relative magnitude of the effects of these two factors two extremes of opinion are illustrated by the statement of Maximov (15) and the work of Porterfield (19, 20). Maximov states (p. 135): "The retarding influence of light on growth is so great that it creates a definite daily period-

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² Research Assistant, Associate Committee on Grain Research.

³ Graduate Assistant.

⁴ Professor of Genetics and Plant Breeding.

icity. During the night, plants grow more rapidly than during the day, in spite of the lower nocturnal temperatures". The statement apparently applies to all plants, the only exception noted having to do with very sharp drops in nocturnal temperatures which result in stunted plants. Porterfield (20), on the other hand, found a consistently higher rate of growth in the bamboo (*Phyllostachys nigra*) during the day than during the night.

In the following sections no attempt is made to give a complete review of the literature, but representative work is cited. It must be noted at the outset that a large proportion of the data pertinent to the present study has been obtained by measuring elongation of a particular organ rather than by measuring increase in total growth as shown by dry weights. Bakhuyzen (1) considers that since elongation is a unidimensional measure, it does not give as accurate an estimation of growth as does dry weight. There is some indication that the two measures do not give the same type of results, at least with particular material. Bakhuyzen, however, considers that measurements of elongation of wheat leaves and internodes yield typical growth curves, although the first part of the curve is usually missed in actual measurements.

The results of all studies show increased growth with increased temperatures (9, 13, 15, 17). For example, Leitch (9) found that the elongation of pea roots immediately after germination was directly dependent on temperature, the curve being a straight line between 12 and 29° C. If this straight line is extrapolated it cuts the line of zero growth at 3° C., while experimental results showed this point to be at -2° C. The minimum temperature at which growth takes place varies with the plant. Values for maize and wheat are given as 5 to 10° C. and 0 to 5° C. respectively (15); and for maize as 49° F. (17). The results of Leitch (9) show that such values must be determined experimentally, since extrapolation from results at higher temperatures does not always give a reliable figure.

The effect of light has not been as definitely determined. Sunlight certainly retards the growth of many plants (6, 8, 10, 14, 18, 21), and it is apparently the shorter wave-lengths that are effective (14, 25). Studies with various species of bamboo have shown that growth of some is retarded during hours of bright sunlight (10, 18), while that of others is not (19, 20).

Many of the studies carried out have been concerned with the relative rate of growth by day and night. There has been no uniformity in the division of the day into these two periods, however, and a fair comparison of results is rendered difficult. In general it must be concluded that the greater growth was obtained at night (10, 12, 14, 16, 18, 21, 24), but definite exceptions to this result have been noted (19, 20). It must further be concluded, however, that the data secured are often inadequate to separate the effects of the various environmental conditions.

Some investigators (2, 10, 11, 16, 24) regard the greater growth by night as a result of moisture relations rather than of the inhibiting effect of sunlight. This conclusion has been reached in various ways, but in few cases has it been substantiated by experiments under controlled conditions or by results sub-

jected to statistical analysis. Loomis (11), however, did get a definite effect when the moisture supply was controlled.

The work of Gregory (6) on the growth of barley is more like the present study than any others with which we are familiar. The measurements of growth used are different, but the results obtained were subjected to a statistical analysis. Partial correlations showed that growth was positively and significantly correlated with day temperatures, but negatively and usually significantly correlated with bright sunlight. Most of the correlation coefficients involving evaporation data were not significant, but Gregory states that the primary data could not be considered satisfactory.

The present study was undertaken with the hope that more definite information might be obtained as to the rate of growth of plants by day and night, and as to the relative importance of the various external factors affecting growth. All data were collected with the intention of using them in a statistical analysis, the results of which should show definitely the relative importance of the various factors under our local conditions.

Material and Methods

The studies reported in this paper were carried out at Edmonton, Alberta, during the summers of 1937 and 1938. The more important measurements were made with maize and gladiolus plants, although in the first year wheat was included in the study. These plants were selected because the stems all possess a well-defined terminal point, which makes accurate measurement relatively easy, they all grow rapidly enough to give growth figures that are high in comparison with the error of reading, and the elongation should be a fair measure of growth, since growth in this case is largely unidimensional. Maximov (15) states that in annual cereals elongation does not begin until all internodes and inflorescences have been laid down, and Bakhuyzen (1) found that the elongation of the two upper internodes takes place at the same time. The "growth" of the wheat plants that we measured was, therefore, the elongation of the cells of the upper internodes of the stems. This is probably true of maize as well, but in gladiolus a somewhat different situation exists. The first elongation results from the lengthening of the flower spike below the first floret. After the spike is well out of the sheath, elongation between florets, beginning at the lowest one, takes place. It seemed possible that this latter elongation might upset the essentially linear relations between elongation and external factors, but such proved not to be the case. As will be seen later, there is no evidence that elongation of the stem as a whole did not proceed very regularly.

Conditions at Edmonton are particularly favourable for separating the effects of temperature and sunlight. Night temperatures are usually considerably lower than those by day, and the total range of temperatures during an experiment is usually high. In this latitude ($53\frac{1}{2}^{\circ}$ N) the maximum possible sunlight during the time most of these measurements were made

is almost 15 hr. a day. The division of the day into three eight-hour periods starting at 4 a.m. gives two daylight periods and one dark period.

The material used in this study and the series designations were as follows:—

Wheat I, 1937. Variety Red Bobs, seed obtained from the pure-seed plots of this Department. Measurements made on 29 plants at 4 a.m., noon and 8 p.m. from July 13 to July 22.

Wheat II, 1937. Variety Red Bobs. Measurements made on 30 plants at the same times from July 19 to July 27.

Maize, 1937. An early sweet hybrid (I_M-34-1), developed in this Department. Measurements made on 24 plants at the same times from July 17 to July 25.

Gladiolus I, 1937. Mixed varieties. Measurements made on 20 plants at the same times from July 22 to August 1.

Gladiolus II, 1937. Mixed varieties. Measurements made on 25 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from August 7 to August 15.

Unshaded maize, 1938. Same variety as in 1937. Measurements made on 30 plants every four hours starting at 4 a.m. from July 19 to July 28.

Shaded maize, 1938. Ten plants in the same plot and for the same periods as those of the preceding series. During the day these plants were shaded on two sides and from above with white cotton cloth. Air movement was but slightly reduced, and air temperatures in the daytime were only about 1° F. higher than in the open. The shades were removed at night.

Gladiolus I, 1938. Variety Bit O' Heaven. Measurements made on 25 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from July 24 to July 30.

Gladiolus II, 1938. Variety Picardy. Measurements made on 20 plants at 4 a.m., 8 a.m., noon, 4 p.m. and 8 p.m. from August 2 to August 10.

The wheat and all gladiolus series were grown in a garden which was unshaded by trees or buildings. The maize was grown in a similar garden in 1937, but in an open field at the University of Alberta in 1938.

The periods of the day are designated as follows: 4 a.m. to 8 a.m., morning; 8 a.m. to noon, early midday; noon to 4 p.m., late midday; 4 p.m. to 8 p.m., evening; and 8 p.m. to 4 a.m., night.

Before measurements were begun, small stakes with squared tops were driven into the ground close to the stem to be measured. All measurements were made with a metre stick, graduated in millimetres, placed squarely on the top of the stake. The tip of the stem (head, tassel or flower spike) was held firmly, but without strain, against the stick and the reading made to the nearest millimetre. Measurements were begun as soon as the tip appeared.

In 1937 no records of temperature, sunlight, etc., were kept for the exact locations at which the plants were grown. The data used are those recorded at the Edmonton station of the Dominion Meteorological Service. In 1938, continuous records were kept in the field in which maize was grown. These

records were compared with those of the Dominion station, and agreed so well that the latter have been used throughout this paper in order to make the results of the two years uniform.

In arriving at the mean temperature and mean relative humidity, hourly readings from continuous records were averaged. Sunlight records were obtained with a Campbell-Stokes sunshine recorder. The data so obtained are inadequate for evaluating the intensity factor of sunlight, but were the best obtainable.

The accuracy of the growth measurements was determined by carrying out an analysis of variance for the data obtained for 10 consecutive readings on 25 plants. The error so determined includes both the personal error of observation and the interaction of individual plant growth with time. The standard error for the mean of 25 plants (*Gladiolus* I, 1938) was 0.056 cm. The corresponding value for the personal error only (determined on the results of four replicate readings made at one time) was 0.022 cm.

Results

Individual data, or even means for individual periods, are much too numerous to include in this paper. The presentation and discussion of the actual data are confined to a minimum, and in the graphs only data from single series are given. These results illustrate general effects, the specific importance of each one being much more clearly determined by the results of the statistical analysis. In this paper, elongation of the stem is termed "growth".

ACTUAL DATA

A summary of the mean growth and temperature data for eight-hour periods in all series is presented in Table I. Growth was in general proportional to temperature, the agreement being closest for maize, and least for *gladiolus*.

TABLE I
MEAN GROWTH AND TEMPERATURE FOR EIGHT-HOUR PERIODS

Series	No. of days	8 p.m. to 4 a.m.		4 a.m. to noon		Noon to 8 p.m.	
		Growth, cm.	Temperature, °F.	Growth, cm.	Temperature, °F.	Growth, cm.	Temperature, °F.
Wheat I, 1937	7	1.08	59.6	1.17	60.8	1.33	67.9
Wheat II, 1937	6	1.07	56.3	1.37	61.0	1.72	69.3
Maize, 1937	7	0.94	58.7	1.60	63.2	2.55	71.6
<i>Gladiolus</i> I, 1937	10	0.99	54.5	1.23	57.3	1.46	66.5
<i>Gladiolus</i> II, 1937	9	0.98	54.0	1.31	57.9	1.18	64.8
Unshaded maize, 1938	9	1.16	58.6	1.47	63.2	2.50	75.8
Shaded maize, 1938	9	1.17	58.6	1.66	63.2	2.62	75.8
<i>Gladiolus</i> I, 1938	6	1.62	56.2	1.66	62.2	2.19	74.4
<i>Gladiolus</i> II, 1938	8	1.18	50.0	1.10	54.0	1.66	60.8

This relation is illustrated in Fig. 1, which shows the results for individual eight-hour periods for the unshaded maize, 1938. Included in this graph are sunlight and relative humidity values. There were only traces of rainfall during the ten days.

There are exceptions to this relation between growth and temperature, however, and the exceptions are greatly accentuated when results for four-hour periods are considered. The results for the four daylight periods are presented in Table II, and in Fig. 2, together with data for sunlight, humidity, and rainfall. These results show a reversal of the general growth-temperature

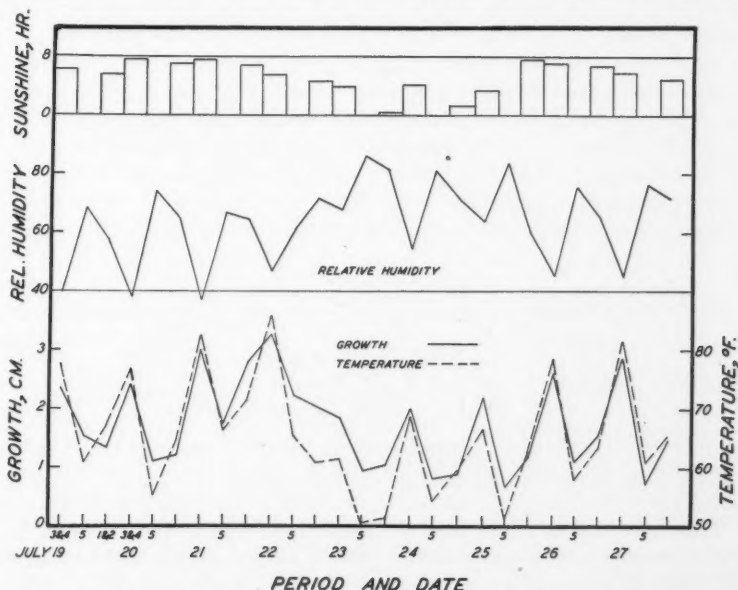


FIG. 1. Unshaded maize, 1938. Growth during 8-hr. periods in relation to external factors. Periods are numbered as follows: 1, morning (4 a.m. to 8 a.m.); 2, early midday (8 a.m. to noon); 3, late midday (noon to 4 p.m.); 4, evening (4 p.m. to 8 p.m.); 5, night (8 p.m. to 4 a.m.).

relation when there was bright sunlight during the middle of the day. When there was no sunshine, however, growth was roughly parallel to temperature. While these effects were much more evident with gladiolus than with maize, the conclusions are applicable to both plants.

Sunlight depressed growth much less during the morning and evening periods than during the middle of the day. The results in Table II and Fig. 2 do not show that sunlight had no effect during these periods, but it is fairly evident from Fig. 3 that only during the midday periods was the depressing effect appreciable. Fig. 3 is for the second gladiolus series, 1938, and the points for each period are clearly defined. The figures beside the points indicate the hours of sunlight per four-hour period. Since no midnight

readings were taken, the points for the night period are plotted as half the total growth against the mean temperature for the eight hours. This method merely reduces the total number of entries, but makes no assumptions regarding the distribution of growth during the two halves of the eight-hour period. The growth for every midday period during which sunlight was recorded was depressed below the general growth-temperature line as determined for the other periods. The growth figures for the midday periods on days when the sun did not shine are in good agreement with those for other periods. Regardless of the duration of direct sunlight, growth during the morning and evening periods was apparently not affected in the way it was during the midday periods. This conclusion will later be shown to be justified by the results of the statistical analysis.

There is an apparent concentration of growth readings for the morning and night periods above (relatively) those for the other periods, regardless of sunlight. Whether this is significant or not cannot be determined from the present data, but it seems possible that the results for the midday and evening periods are affected by some factor not accounted for in this work. This conclusion is supported by the position of the two regression lines shown

TABLE II
MEAN GROWTH, TEMPERATURE AND SUNLIGHT DURING THE 4-HOUR DAYLIGHT PERIODS

Series	No. of days	Morning (4 a.m. to 8 a.m.)			Early midday (8 a.m. to 12 noon)		
		Growth, cm.	Temperature, °F.	Sunlight, hr.	Growth, cm.	Temperature, °F.	Sunlight, hr.
Gladiolus II, 1937	9	0.73	52.8	1.5	0.48	63.1	2.3
Unshaded maize, 1938	9	0.52	55.6	2.0	0.95	70.8	3.0
Shaded maize, 1938	9	0.49	55.6	2.0	1.17	70.8	3.0
Gladiolus I, 1938	6	0.93	55.2	1.9	0.73	69.2	2.8
Gladiolus II, 1938	8	0.65	48.9	1.3	0.45	59.0	2.1
Series	No. of days	Late midday (12 noon to 4 p.m.)			Evening (4 p.m. to 8 p.m.)		
		Growth, cm.	Temperature, °F.	Sunlight, hr.	Growth, cm.	Temperature, °F.	Sunlight, hr.
Gladiolus II, 1937	9	0.37	66.9	2.5	0.81	62.7	1.2
Unshaded maize, 1938	9	1.02	78.2	3.5	1.48	73.5	2.1
Shaded maize, 1938	9	1.39	78.2	3.5	1.24	73.5	2.1
Gladiolus I, 1938	6	0.74	77.2	2.9	1.45	71.6	1.7
Gladiolus II, 1938	8	0.68	63.5	1.4	0.97	58.2	0.9

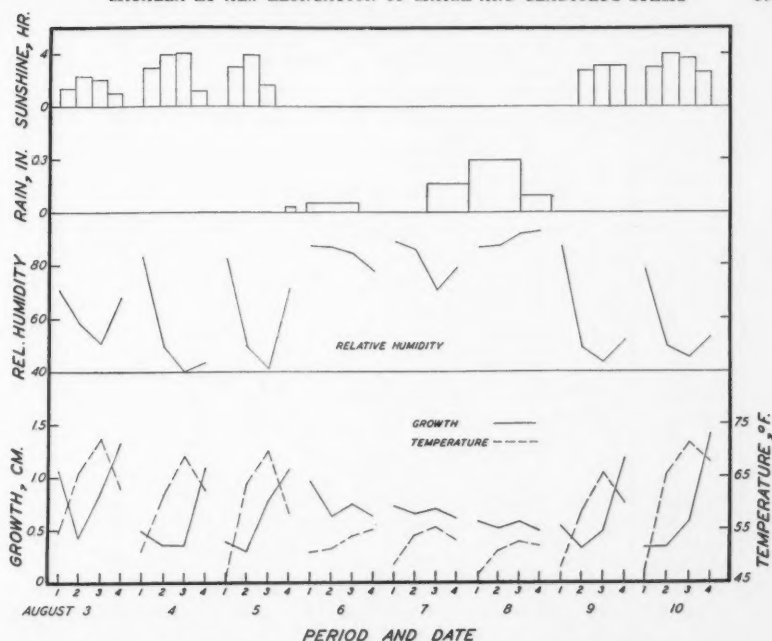


FIG. 2. *Gladiolus* II, 1938. Growth during 4-hr. daylight periods in relation to external factors. Periods are numbered as in Fig. 1.

(anticipated from the data and discussion in the next section). Allowing for the effect of sunlight did not account for the entire difference in the relative levels of growth during the midday periods as compared with the others. This may be the result of inadequate sunlight data or increased error in temperature data at the higher levels. Certainly it would be expected that the temperatures of the meristems would be further from air temperatures on a bright warm day than on a cool one. If the temperatures recorded during sunny hours were higher than the temperatures of the meristems, the effect would be as shown in Fig. 3.

A number of other factors may have been operative in determining the magnitude of the midday readings, but since it is impossible to determine which were important, extended discussion does not seem warranted.

The failure of the two regression lines to coincide when the effect of sunlight was removed raises a question with regard to the linearity of the effects of temperature and sunlight on growth rate. The data obtained with Shaded Maize, 1938, and with *Gladiolus* II, 1938 (the series shown in Fig. 3) were tested for non-linearity. The deviations from the linear regression line were not significant for either the growth-temperature or growth-sunlight relation. Thus the failure to get better agreement between the two regression lines is probably due to deficiencies in the data or to some factor not here considered.

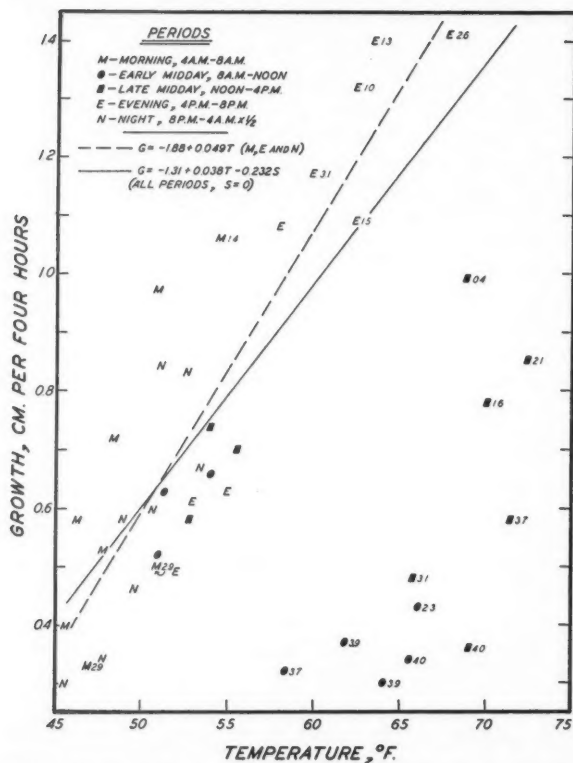


FIG. 3. *Gladiolus* II, 1938. The effects of temperature and sunlight on growth per 4 hr. The figures beside the symbols denote hours of sunlight per 4-hr. period.

Whether 8 a.m. and 4 p.m. mark the extremes of time at which sunlight exerts a depressing effect on growth is doubtful. Measurements were made at 6 p.m. and 10 p.m. in addition to the regular times on July 26 (*Gladiolus* I, 1938). The results on the basis of hourly growth, together with temperature and sunlight values, are given in Table III. These data can be considered to

TABLE III
MEAN HOURLY GROWTH OF *GLADIOLUS* FROM NOON, JULY 26 TO 4 A.M., JULY 27

Time	Growth, cm.	Temperature, °F.	Sunlight, hr.
12 noon to 4 p.m.	0.12	80.5	0.95
4 p.m. to 6 p.m.	0.24	81.0	0.95
6 p.m. to 8 p.m.	0.58	72.5	0.70
8 p.m. to 10 p.m.	0.30	65.0	—
10 p.m. to 4 a.m.	0.21	56.2	—

give only an indication, but they suggest that the depressing effect of sunlight may be felt later than 4 p.m. Presumably the same effect might have been apparent during the morning period. Further discussion is hardly warranted here, since only one day's results are available.

STATISTICAL ANALYSIS OF THE DATA

The methods of statistical analysis used in the following sections are fully outlined by Goulden (5) and Snedecor (23). The dependent variable is always growth (g , the increase in length), and the independent variables studied are temperature (t), sunlight (s), and relative humidity (h). Rain-fall is omitted from the calculations, since there was none during the time several of the series were studied, and because preliminary examination showed that this factor was not of primary importance. The number of periods represented for each series and each time is given in Table IV.

TABLE IV

THE NUMBER OF INDIVIDUAL PERIODS REPRESENTED FOR EACH SERIES AND EACH TIME

Series	All periods	Daily totals	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Wheat I, 1937	—	7	—	—	8
Wheat II, 1937	—	6	—	—	7
Maize, 1937	—	7	—	—	8
Gladiolus I, 1937	—	10	—	—	11
Gladiolus II, 1937	45	8	18	9	8
Maize, 1938	54	9	20	9	9
Shaded maize, 1938	54	9	20	9	9
Gladiolus I, 1938	31	6	12	6	6
Gladiolus II, 1938	42	8	17	9	8

Temperature Effects

The simple and partial correlation coefficients obtained with the temperature data for all series are presented in Table V. All the simple coefficients are positive and most of them significant. There is, however, a consistency in those that are not significant, since all but two of them are for gladiolus, and most of them are for data that include results of the midday periods. Holding the effects of sunlight and humidity constant in general increased the correlation between growth and temperature, and brought the results for gladiolus into agreement with those for wheat and maize.

Gladiolus I, 1938, gave lower partial correlation coefficients than did any other series. Measurements of this series were carried out for only six days, and variations in external conditions during this time were much less than with most of the other series. More extended measurements would probably have made the results for this series significant.

The effect of temperature on the two series of maize plants grown in 1938 was similar. The only difference in the two series was that the shaded plants

TABLE V

RELATION BETWEEN TEMPERATURE AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods	Daily totals	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
<i>Simple correlation coefficients, r_{gt}</i>					
Wheat I, 1937	—	0.821*	—	—	0.824**
Wheat II, 1937	—	0.865*	—	—	0.723
Maize, 1937	—	0.987**	—	—	0.921**
Gladiolus I, 1937	—	0.939**	—	—	0.929**
Gladiolus II, 1937	0.256	0.780*	0.069	0.811**	0.613
Maize, 1938	0.751**	0.891**	0.548*	0.859**	0.782*
Shaded maize, 1938	0.897**	0.863**	0.717**	0.810**	0.638
Gladiolus I, 1938	0.152	0.588	0.000	0.346	0.829*
Gladiolus II, 1938	0.289	0.670	0.067	0.941**	0.838**
<i>Partial correlation coefficients, r_{gt-h}</i>					
Wheat I, 1937	—	0.722	—	—	0.831 ^a
Wheat II, 1937	—	0.882*	—	—	0.726 ^a
Maize, 1937	—	0.995**	—	—	0.921** ^a
Gladiolus I, 1937	—	0.940**	—	—	0.935** ^a
Gladiolus II, 1937	0.823***	0.791*	0.736***	0.747 ^b	—
Maize, 1938	0.602**	0.909**	0.639**	0.908**	-0.321 ^a
Shaded maize, 1938	0.620**	0.725*	0.586*	0.883**	-0.071 ^a
Gladiolus I, 1938	0.340	0.433	0.937**	0.694	0.666 ^a
Gladiolus II, 1938	0.647**	0.872*	0.808**	0.826*	0.841 ^a

^a Coefficient r_{gt-h} , as there was no sunlight during these periods.

^b Humidity data not available.

*Significant beyond the 5% point.

**Significant beyond the 1% point.

were protected from the direct sun by light white cotton. The results for the night period (8 p.m. to 4 a.m.) with the two series are not in agreement with the others in Table V. The negative partial correlation coefficients cannot be accepted as a true indication of the effect of temperature on growth, and must be attributed to some peculiarity of the data involved.

The general conclusion reached as a result of the correlation analyses is that there is a significant positive effect of temperature on the growth of these three plants, and that in many instances this effect accounts for a large proportion of the variability found in the growth rates.

Sunlight Effects

The simple and partial correlation coefficients involving the sunlight data for all series are presented in Table VI. It should be emphasized again that when all periods are considered together, the sunlight data for midday periods only were included in the correlation analysis.

Only a few of the simple correlation coefficients are significant, but they exhibit a fairly consistent behaviour. For most of the gladiolus series the

TABLE VI

RELATION BETWEEN HOURS OF SUNLIGHT AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods ^a	Daily totals	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.
<i>Simple correlation coefficients, r_{gs}</i>				
Wheat I, 1937	—	0.580	—	—
Wheat II, 1937	—	0.675	—	—
Maize, 1937	—	0.205	—	—
Gladiolus I, 1937	—	0.352	—	—
Gladiolus II, 1937	-0.398**	0.228	-0.331	0.474
Maize, 1938	0.157	0.525	0.154	0.286
Shaded maize, 1938	0.615**	0.681*	0.567	0.394
Gladiolus I, 1938	-0.542**	-0.414	-0.951**	-0.330
Gladiolus II, 1938	-0.415**	-0.178	-0.667**	0.695*
<i>Partial correlation coefficients, $r_{gs \cdot th}$</i>				
Wheat I, 1937	—	-0.150	—	—
Wheat II, 1937	—	0.721	—	—
Maize, 1937	—	-0.809	—	—
Gladiolus I, 1937	—	-0.390	—	—
Gladiolus II, 1937	-0.842***	-0.321 ^b	-0.769***	0.000 ^b
	-0.600**	-0.624	-0.030	-0.163
Maize, 1938	—	—	—	—
Shaded maize, 1938	0.163	0.001	0.345	0.414
Gladiolus I, 1938	-0.776**	-0.160	-0.995**	-0.898*
Gladiolus II, 1938	-0.841**	-0.692	-0.944**	-0.063

^a Sunlight during the midday periods only.^b Humidity data not available.

*Significant beyond the 5% point.

**Significant beyond the 1% point.

coefficients are negative, while for maize they are all positive, though not significant. The significant effect of sunlight on the growth of Shaded Maize, 1938, disappears when the effects of temperature and humidity are removed. Thus sunlight affected growth of these shaded plants only through its effect on the other environmental conditions.

The partial correlation coefficients are consistently negative except for the daily totals for one wheat series. This result cannot be explained, but it is not significant. The results show that the depressing effect of sunlight on growth was more pronounced with gladiolus than with maize. They likewise show that only for those analyses in which results for midday periods were included was this depressing effect significant. There is one exception with Gladiolus I, 1938, but even though the negative correlation coefficient was significant, the regression coefficient was much smaller than that for the midday periods.

The effect of sunlight on daily growth was negative but not significant. Since the number of days involved was small, the coefficients must be very

high to be significant, and more extended series would probably show a significant depressing effect of sunlight on total daily growth. This conclusion is supported by the results for shaded maize plants, which on bright sunny days made an average growth of 6.6 cm. per day as compared with 5.8 cm. for the unshaded plants. This difference is significant, and cannot be attributed to temperature differences, since temperature under the shade was not more than 1° F. higher than the temperature in general.

These results show that bright sunlight exerts a depressing effect on growth during the midday periods, and that this effect is more pronounced with gladiolus than with maize. They do not permit a final conclusion as to the effects during other periods, but these, if any, are small compared with the effects during the middle of the day. There is a strong indication that total daily growth is also depressed by bright sunlight.

Humidity Effects

The simple and partial correlation coefficients involving humidity data are presented in Table VII. Very little need be said regarding these results, since only one of the partial coefficients is significant.

TABLE VII

RELATION BETWEEN RELATIVE HUMIDITY AND GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY SIMPLE AND PARTIAL CORRELATION COEFFICIENTS

Series	All periods	Daily means	8 a.m. to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
<i>Simple correlation coefficients, r_{gh}</i>					
Wheat I, 1937	—	-0.484	—	—	-0.226
Wheat II, 1937	—	-0.889*	—	—	0.032
Maize, 1937	—	-0.621	—	—	-0.378
Gladiolus I, 1937	—	0.170	—	—	0.066
Gladiolus II, 1937	—	—	—	—	—
Maize, 1938	-0.656**	-0.674*	-0.225	-0.475	-0.902**
Shaded maize, 1938	-0.829**	-0.689*	-0.545	-0.437	-0.710*
Gladiolus I, 1938	0.003	0.105	0.754	-0.375	-0.662
Gladiolus II, 1938	-0.161	-0.130	0.165	-0.794*	-0.045
<i>Partial correlation coefficients, $r_{gh \cdot ts}$</i>					
Wheat I, 1937	—	0.023	—	—	0.289
Wheat II, 1937	—	-0.018	—	—	-0.099
Maize, 1937	—	0.182	—	—	0.463
Gladiolus I, 1937	—	0.107	—	—	0.280
Gladiolus II, 1937	—	—	—	—	—
Maize, 1938	0.011	0.502	0.375	0.502	-0.756*
Shaded maize, 1938	0.099	0.452	0.256	0.672	-0.409
Gladiolus I, 1938	-0.221	0.121	0.384	-0.620	0.040
Gladiolus II, 1938	-0.080	-0.027	-0.083	-0.170	0.139

*Significant beyond the 5% point.

**Significant beyond the 1% point.

The significant simple correlation coefficients are all negative and are significant only because humidity was highly and negatively correlated with temperature. When the effect of temperature was removed the relation between growth and humidity becomes negligible in most series.

The only significant partial coefficient is also negative. This result cannot be accepted as biologically significant, and since it is for the same series as that which yielded the negative relation between growth and temperature, the peculiarity of the data is emphasized.

In the present study there is no evidence that relative humidity has any effect in determining the rate of growth. However, since this conclusion applies to fluctuations in relative humidity independent of those associated with temperature differences, it is possible that real effects of atmospheric humidity may have been confounded with temperature effects.

Multiple Correlation Coefficients

The multiple correlation coefficients summarizing the combined effects of temperature, sunlight, and humidity are given in Table VIII. Most of these are highly significant and show that when results for all periods are considered together (sunlight for midday periods only), from 65 to 80% of the variability in growth can be accounted for in terms of temperature, sunlight, and humidity. For individual periods and daily means an even greater proportion of the variability in growth results is accounted for.

TABLE VIII

COMBINED EFFECT OF TEMPERATURE, SUNLIGHT,^a AND RELATIVE HUMIDITY ON THE GROWTH OF WHEAT, MAIZE, AND GLADIOLUS AS MEASURED BY MULTIPLE CORRELATION COEFFICIENTS

Series	All periods R_{g-ts}	Daily means R_{g-ts}	8 a.m. to 4 p.m. R_{g-ts}	4 p.m. to 8 p.m. R_{g-ts}	8 p.m. to 4 a.m. R_{g-ts}
Wheat I, 1937	—	0.825	—	—	0.840*
Wheat II, 1937	—	0.990**	—	—	0.727
Maize, 1937	—	0.996**	—	—	0.937**
Gladiolus I, 1937	—	0.997**	—	—	0.935**
Gladiolus II, 1937	0.854** ^b	0.803 ^b	0.769** ^b	0.784 ^b	—
Maize, 1938	0.855**	0.933**	0.661*	0.933*	0.913**
Shaded maize, 1938	0.900**	0.864	0.761**	0.907*	0.711
Gladiolus I, 1938	0.803**	0.872	0.995**	0.916	0.830
Gladiolus II, 1938	0.861**	0.914*	0.969**	0.941**	0.841*

^a Midday periods only.

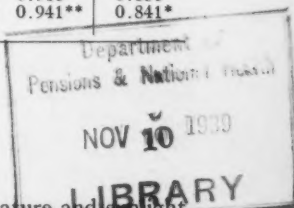
^b R_{g-ts} , humidity values not available.

*Significant beyond the 5% point.

**Significant beyond the 1% point.

Regression of Growth on Temperature and Sunlight

The partial regression coefficients for growth on temperature and sunlight for all series and periods are presented in Table IX. The results for all periods



together are the most reliable since the number of entries for individual periods is small, and one result which was not in agreement with the general trend could, and in some cases did, alter the regression coefficients unduly.

TABLE IX
PARTIAL REGRESSION COEFFICIENTS OF GROWTH OF WHEAT, MAIZE, AND GLADIOLUS ON
TEMPERATURE AND SUNLIGHT

Series	All periods cm./4 hr./ °F.	Daily totals cm./day/ °F.	8 a.m. to 4 p.m. cm./4 hr./ °F.	4 p.m. to 8 p.m. cm./4 hr./ °F.	8 p.m. to 4 a.m. ^a cm./8 hr./ °F.
<i>Growth on temperature, b_{gt-tk}</i>					
Wheat I, 1937	—	0.230	—	—	0.059
Wheat II, 1937	—	0.151	—	—	0.074
Maize, 1937	—	0.261	—	—	0.079
Gladiolus I, 1937	—	0.230	—	—	0.078
Gladiolus II, 1937	0.035 ^b	0.158 ^b	0.030 ^b	0.039 ^b	0.083 ^b
Maize, 1938	0.044	0.312	0.023	0.044	0.069 ^c
Shaded maize, 1938	0.039	0.196	0.030	0.042	0.059 ^c
Gladiolus I, 1938	0.027	0.114	0.019	0.011	0.111
Gladiolus II, 1938	0.038	0.300	0.025	0.064	0.123
<i>Growth on sunlight, b_{gt-sk} (cm. per hr. sunshine)</i>					
Wheat I, 1937	—	-0.040	—	—	—
Wheat II, 1937	—	0.081	—	—	—
Maize, 1937	—	-0.055	—	—	—
Gladiolus I, 1937	—	-0.041	—	—	—
Gladiolus II, 1937	-0.206 ^b	-0.032 ^b	-0.090 ^b	0.000 ^b	—
Maize, 1938	-0.049	-0.169	-0.051	-0.005	—
Shaded maize, 1938	0.007	-0.003	0.069	0.146	—
Gladiolus I, 1938	-0.208	-0.035	-0.105	-0.030	—
Gladiolus II, 1938	-0.232	-0.125	-0.153	-0.003	—

^a b_{gt-tk} since there was no sunlight at night.

^b b_{gt-tk} and b_{gt-sk} since humidity data not available.

^c simple regression coefficients.

The temperature effect was fairly uniform, an increase of 1° F. increasing the growth per four hours by about 0.04 cm. The results for daily totals are for 24 hr., and when reduced to a four-hour basis agree reasonably well with the results for all periods. Similarly the results for the night periods (8 p.m. to 4 a.m.) are for eight hours, and agree well on the four-hour basis except for the last two series, which are higher than the other coefficients obtained.

The correlation results were subjected to a covariance analysis, splitting the correlation into within and between periods. With only one series was there any significant difference between the regression coefficients for the different periods, and this difference disappeared when one irregular value

was omitted. It is concluded, therefore, that the correlation involving temperature is homogeneous for all periods.

There is a pronounced variability in the regression coefficients involving sunlight, however, the results for the evening period yielding insignificant coefficients while those for all periods and for midday periods are relatively high, particularly for the gladiolus. Bright sunlight for four hours during the middle of the day depressed the growth of gladiolus approximately 0.8 cm. below that expected at any specified temperature. This means that the temperature had to be about 24° F. higher during periods of bright sunlight than during the other periods to give the same increase in growth. The corresponding figure for maize is about 4.5° F.

The fact that the regression coefficients for the midday periods alone are lower than those for all periods requires an explanation. The magnitude of these coefficients depends not only on the degree of correlation, but also on the magnitude of the standard deviations of growth and sunlight readings. During the midday periods alone, the variability in growth rates was low, because warm days were sunny and cool days were not. For example, with *Gladiolus* II, 1938, the standard errors for growth during midday and all periods were respectively 0.203 and 0.959, while those for temperature during the same periods were 7.52 and 7.96, and those for sunlight were 1.41 and 1.60. Thus, even if the correlations were higher during midday periods the regression coefficients would be lower than those for all periods.

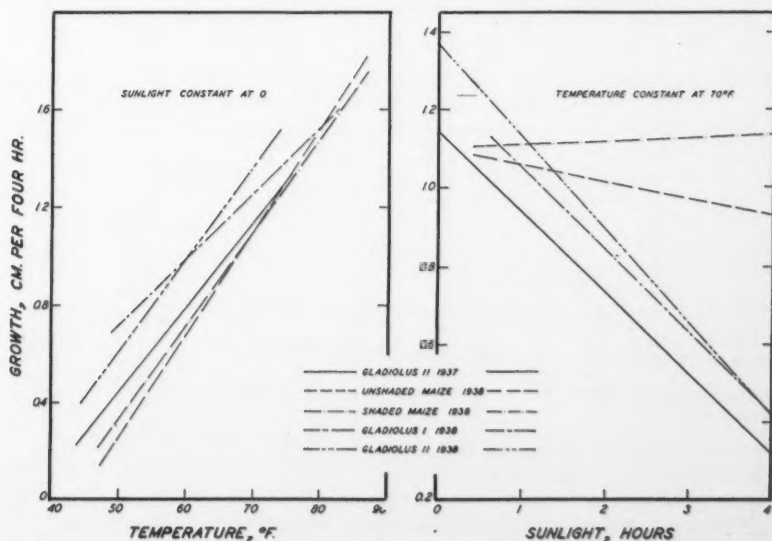


FIG. 4. Regression of growth on temperature and sunlight. Partial regressions holding the second independent variable constant. Results for all individual periods. Lines cover only the temperature and sunlight ranges actually obtained experimentally.

The general independent effects of temperature and sunlight (sunlight effects for midday periods only) are shown in Fig. 4. These two graphs show the growth expected as expressed in terms of temperature or sunlight under constant conditions of the other independent variable. Under any other specific conditions than those shown, for example sunlight constant at four hours or temperature constant at 60° F., the slope of each line, and the relation among all lines would be unaltered, but the numerical value of the growth increases would be changed.

TEMPERATURE COEFFICIENTS

Many biological reactions do not obey the van't Hoff law with respect to increased rates with increasing temperature. The work of Leitch (9) has already been referred to, and while he did not calculate Q_{10} values, it can readily be seen that his results would yield steadily falling values as temperature increased. Only at about 13 to 23° C. was this value equal to 2.0. The present study offered an opportunity to calculate Q_{10} values at various levels. The results of such calculations for the temperature increase from 15 to 25° C., based on partial regression coefficients, are presented in Table X. Sunlight and humidity values are taken equal to the averages obtained for each series. Where extrapolation was necessary to obtain a value for 25° C. the result is marked with an asterisk. There is no real difference between the values obtained from series in which no extrapolation was necessary and those in which it was. The mean Q_{10} for the former is 2.04 and for the latter 1.98.

TABLE X

TEMPERATURE COEFFICIENTS FOR THE TEMPERATURE INCREASE 15 TO 25° C. BASED ON PARTIAL REGRESSION COEFFICIENTS

Series	All individual periods	Daily totals	4 p.m. to 8 p.m.	8 p.m. to 4 a.m.
Wheat I, 1937	—	2.31	—	1.98
Wheat II, 1937	—	1.88*	—	2.00*
Maize, 1937	—	2.01	—	2.39
Gladiolus I, 1937	—	2.08*	—	2.04*
Gladiolus II, 1937	1.84	—	2.03	2.07*
Unshaded maize, 1938	2.22	2.14	1.90	1.97*
Shaded maize, 1938	2.10	1.83	2.12	1.82*
Gladiolus I, 1938	—	—	—	2.11*
Gladiolus II, 1938	1.72	1.73*	2.15*	1.97*

*Involves extrapolation beyond temperatures actually obtained experimentally.

The effect of temperature on the temperature coefficients for Unshaded Maize, 1938, at different periods of the day is illustrated in Fig. 5. The range

covered by these curves involves no extrapolation of regression lines. In each instance (except 8 p.m. to 4 a.m.) the partial regression coefficient has been used, the other independent variables being held constant at the mean value obtained experimentally. The variations at lower temperatures are due to the differences in the point at which no growth is calculated to take place. At higher temperatures the agreement is excellent.

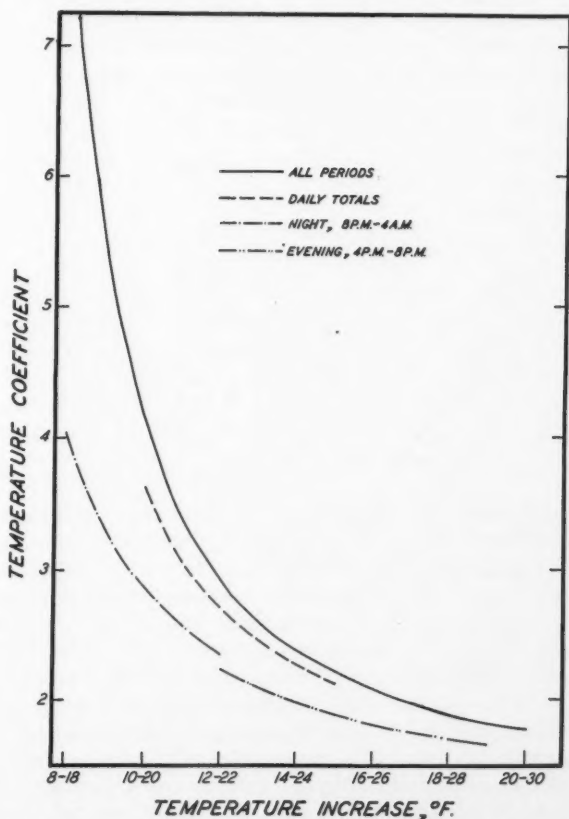


FIG. 5. Unshaded maize, 1938. Temperature coefficients in relation to temperature level.

It has frequently been remarked, of course, that since growth is not a single reaction it cannot be expected to obey any simple rule. It is not implied in this discussion that the Q_{10} values express the effect of temperature on a single reaction, but rather on the whole complex mechanism that results in stem elongation. That the values obtained should be as consistent as they are is remarkable when the enormous variations in other factors are considered.

Approximate Minimum Temperature at Which Growth Took Place

By extrapolation of the regression lines for growth in terms of temperature, a measure of the minimum temperature at which growth took place can be obtained. The values for all data with which significant correlation coefficients were obtained are given in Table XI. Again the other variables have been held constant at the means obtained experimentally.

TABLE XI
ESTIMATED MINIMUM TEMPERATURES ($^{\circ}$ F.) AT WHICH GROWTH TOOK PLACE. BASED ON
PARTIAL REGRESSION COEFFICIENTS

Series	All periods	Daily totals	Evening period	Night period
Wheat I, 1937	—	45.4	—	40.6
Wheat II, 1937	—	37.6	—	—
Maize, 1937	—	41.2	—	40.5
Gladiolus I, 1937	—	42.3	—	41.7
Gladiolus II, 1937	37.6	35.7	41.6	—
Unshaded maize, 1938	44.3	43.2	39.1	40.5
Shaded maize, 1938	41.6	37.1	42.9	—
Gladiolus I, 1938	22.8	—	—	42.8
Gladiolus II, 1938	34.2	39.3	43.3	40.4

It has already been noted that such extrapolation is invalid in determining the exact level at which growth takes place. The relative uniformity of the results in Table XI, however, indicates that there is a distinct similarity in the behaviour of the three plants studied. The results for all periods should be the most reliable, since they are obtained from the most extensive data. They likewise involve the least extrapolation except for the night periods with which the same amount would be required. These values indicate that the gladiolus will grow at a lower temperature than will maize. The result for Gladiolus II, 1938, is low because the regression coefficient is low. This flattens the regression line with a consequent greater extension being necessary before it cuts the growth axis. Although none of these values can be considered exact, they are in fairly good agreement with those reported by others (15, 17). The results obtained with maize are a few degrees lower than others reported, but there may be a difference with different varieties.

Discussion

The results presented in this paper show conclusively that the rate of elongation of stems of maize, gladiolus, and wheat is affected by atmospheric temperature and sunlight. With most of the series, the variations in temperature and sunlight during individual periods account for from 60 to 90%

of the variability in growth. The effect of humidity is less well defined, but it appears to be relatively unimportant under the conditions of our experiments. All of these plants had an adequate supply of moisture during the time measurements were taken, and it seems possible that the humidity might be more important under conditions of moisture deficiency.

It seemed possible that a closer association between growth and external factors might be obtained if an allowance for delayed effect were made. Growth for individual periods was correlated with temperatures for the preceding as well as for the actual periods, that is allowing a four-hour lag in growth, but in general the association was not improved by this procedure. The calculations were repeated allowing a two-hour lag with the same result. It is concluded, therefore, that there is a fairly rapid response to changes in external temperature. The same general result was obtained with sunlight.

One of the primary objects of this study was to determine whether the plants grew faster by day or by night. This question can only be answered by specifying the external conditions. Undoubtedly if the day were bright and sunny, growth of gladiolus from 6 a.m. to 6 p.m. would be less than from 6 p.m. to 6 a.m. unless the temperature during the latter period was relatively low. On the other hand, on a dull day growth from 6 a.m. to 6 p.m. would be greater if the mean temperature were higher. For many of the series, the amount of elongation which would take place under specific conditions could be accurately calculated from a knowledge of temperature and sunlight values. Thus with Gladiolus II, 1938, elongation during the night (dark period) was as great at 60° F. as it was during the midday periods (with continuous bright sunshine) at 84° F.

If, however, we consider the hourly rate of growth during daylight (4 a.m. to 8 p.m.) and during darkness, the former was always the higher. The daylight hours include the evening period, during which actual growth was usually most rapid, since temperature was usually high and sunlight apparently ineffective. There was a sufficient decrease in temperature at night to more than offset the fact that there was no retarding effect of light. In other words, the total retarding effect of light from 4 a.m. to 8 p.m. was less than the retarding effect of the lower temperatures at night.

The effect of sunlight on gladiolus was much more pronounced than that on maize. Nevertheless, the shading experiment with maize showed that growth was greater for the shaded plants, both during the bright-light periods and for the daily totals. The differences could not be due to higher temperature under the shades, since this was never more than 1° higher than in the open.

The results of this study are concerned with the effects of external factors. Although air temperatures should be more or less closely related to the temperature of the meristems, the latter should give a closer association with growth. The relation of sunlight to growth is quite different, since the internal expression of this external factor is not accounted for. It is the light of short wave-length that is active in depressing growth (14, 25), and the difference

in behaviour of different plants might be the result of a difference in the ease of penetration of the waves concerned to the meristems. This is particularly likely if the retarding effect of the light is due to a reduction of sensitivity of cells to auxin action (25).

The results obtained by Gregory (6) are of interest to us because his measures of growth more nearly fulfil the conditions specified by Bakhuyzen (1) as essential for a determination of real growth rates than do ours. The results of his studies with relative growth rates of leaves agree with the results of the present study, although his correlation coefficients were in general lower. It seems likely, therefore, that the conclusions reached as a result of this study may be applicable to growth in general, as well as to stem elongation.

The results here presented offer a possible explanation of some of the experimental results obtained by other workers. Lock (10) considered the day period as between 7.30 a.m. and 5.30 p.m., and found that the growth rate of bamboo was much greater by night than by day. There is every reason to believe that this division of the day would result in an exaggerated difference between night and day growth, since the early and late hours of sunlight, during which growth may be rapid, are excluded from the day period. Of course, the exaggeration would be much less in the tropics, where Lock's work was done, than in northern latitudes. Lock likewise correlated growth with rainfall, but it seems possible that the relation between growth and sunlight might have been just as important. There would be no direct sunlight when it was raining, and if temperature remained constant, growth should be greater when the sun was not shining.

Prescott (21) found two maxima in the rate of growth of maize. These are of interest because they occurred just after sunrise and just before sunset, and our results indicate that similar maxima would have been obtained with hourly measurements. MacDougal (12) found retardation in the growth rate of wheat and maize occurred after 11 a.m. In the present study it began before this, although the exact point cannot be determined from the data obtained. The results obtained by MacDougal may well have been due to sunlight, although he does not mention this factor.

Hanna (7) obtained positive correlations between growth of maize and sunflowers and temperature, and between growth and hours of sunshine. Measurements were made at two- or three-day intervals and only simple correlation coefficients were calculated. Some of Hanna's conclusions are outside the field of this paper, but it seems probable that partial correlations would have shown that the positive relation between growth and sunlight was due to the relation between sunlight and temperature.

Extremes of altitude affect growth of plants, owing partly to temperature differences but also to light effects (15). It seems probable that if not only the duration and intensity, but also the composition of the sunlight were known, a more complete explanation of results obtained could be made. It seems probable that during sunlight hours much less ultraviolet light

would be incident on plants when the atmosphere was moist than when it was relatively dry. If conditions could be controlled so that relative humidity could be varied independently of temperature and sunlight, it might be shown to be more important than it now appears to be.

Controlled environmental conditions are essential for the most accurate determination of the effects of individual factors on the rate of growth. Under such conditions, the effect of light of varying composition could be studied, as could any one factor under constant conditions of others. Unless facilities are available for such control, however, it is believed that the statistical analysis used in this paper offers the most precise method available for separating the effects of the various factors involved in determining growth rates.

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THE DETECTION OF ABNORMAL COW'S MILK BY MICROSCOPIC METHODS¹

BY SEYMOUR HADWEN² AND RONALD GWATKIN³

Abstract

A large number of milk samples from dairy cows have been studied microscopically and culturally, the object being to estimate the numbers of body cells and leucocytes present.

The Breed and sediment counts are compared. The latter is preferred because it furnishes more information than mere enumeration of the cells. In a sediment smear a complete picture of conditions in the udder may be visualized, including the various cellular responses to the organisms causing mastitis. Estimates of the numbers of cells present are sufficient for a diagnosis when accompanied by the information gained in examining the smear.

Differential counts were made for special purposes only, such as to define normality in milk. In defining normal milk, a standard was set which did not tolerate the presence of micro-organisms or polymorphonuclear leucocytes in the samples. In a herd of 60 cows, milk from 9 young cows met this standard. Diphtheroids occurred in 70% of the cows. In comparison with the clean cows, the diphtheroid carriers had larger numbers of polymorphonuclear leucocytes.

In staphylococcal mastitis the leucocytes are often very numerous, and this may persist for months. Staphylococcal infections cause a great influx of large, ring-shaped polymorphonuclear leucocytes. The cocci can generally be found on the smears. Sometimes, when leucocytes are scarce, cocci occur in very large numbers.

In streptococcal mastitis the mononuclear leucocytes are numerous. Loose, irregular clumping is commonly seen, and the polymorphonuclear leucocytes often clump separately. Leucocytes are not as numerous as in staphylococcal cases, but the percentage of the mononuclear leucocytes is higher. Tables showing the increase and decrease of the leucocytes indicate that when they are numerous the infective organisms may be scarce or absent, the reverse being also true. Streptococcal mastitis is sometimes a difficult disease to diagnose microscopically, on account of scarcity of organisms. Various ways of finding the cocci are discussed.

The diagnosis of *B. coli* and *Corynebacterium pyogenes* mastitis is described. Both these organisms may cause severe lesions, and consequently the smears reveal extensive degenerative changes in the leucocytes. The organisms are present in large numbers. *B. coli* and *C. pyogenes* infections produce a different leucocytic picture under the microscope than do pyogenic cocci. The effect of these infections is generally more destructive.

A study of phagocytosis in diseases of the udder furnishes valuable aid in determining the degree of resistance to infection on the part of the host.

Red blood corpuscles in milk may be unaccompanied by any signs of infection. Chromatin-staining granules in milk are caused by degenerative changes in the polymorphonuclear cells. Calcium calculi are found both in the tissues and in the milk. A study of over 20 samples of colostrum is recorded.

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² Director, Department of Pathology and Bacteriology.

³ Research Fellow.

Introduction

The present study relates principally to the different kinds of cells and organisms encountered in milk, and their functions and significance. An examination was made of the milk of 1700 cows, which figure includes the retesting of some of the herds under observation. The results of the microscopic examination were compared with cultural tests carried out simultaneously. These results, together with the percentages of the various organisms encountered, were published in 1937 (5) and will be only briefly referred to here.

Sections of udders have been cut, for the purpose of comparing the cells found in the ducts and alveoli of the udder and those that occur in the milk, and this comparison showed that the cells appear to be identical. Pathological changes accompanying disease have been studied. The above studies naturally bear on the diagnosis of mastitis through microscopic examination. Our principal aim has been to find a method of diagnosis that could be used by practising veterinarians.

The Significance of Leucocytes in Milk

The three volumes compiled by E. Munch-Petersen in Australia have given us much valuable information on mastitis (11). In the words of the late Dr. J. A. Gilruth: "It affords us the most complete résumé on the work accomplished up to the present time".

The following quotations, drawn from the work of investigators in four different countries, do not necessarily represent the opinions held in those countries, but they are the results of well-considered and extensive investigations.

Minett, Stableforth, and Edwards (9) state: "The microscopic examination of centrifuged milk sediment for streptococci is much less reliable than the cultural method. In individual cases, or when small numbers of samples have to be examined, the method may give useful results, because if these are positive, cultural work may be rendered unnecessary."

"The microscopic examination of cream smears to obtain information as to the type and number of cells present is a simple and easily applied method which may serve to direct attention to abnormalities in the gland. As would be expected, however, there are a number of border-line cases in which a correct interpretation is difficult, and the method gives no definite clue to the species of bacteria responsible."

The quotations show that these authors do not favour the examination of either milk sediment or cream under the microscope as a means of diagnosing mastitis. They give figures showing that they were unable to detect streptococci in 47.5% of the cases examined.

Our figures published elsewhere (5) show that we were able to detect 75% of streptococcic cases, and 17% that showed numbers of leucocytes but no streptococci. A further 7.5% showed nothing. These cases were reported

before we had completed our field studies. If other forms of mastitis were included with the streptococcic cases, the results would be even better. It should be pointed out that Minett *et al.* did not mention the types of cells they encountered, while we have found this information valuable as an indication of various responses to infection. The stains they used would preclude to a considerable extent the determination of certain types of cells. Also, examination often reveals whether the leucocytes are combating infection by their phagocytic action, or whether the infection is gaining, as indicated by few leucocytes and free organisms in the milk.

Bourgeois (2) has devoted much time to the study of leucocytes in milk. He advises heating the milk to 70° C. before centrifuging. Giemsa's stain is used. Referring to polymorphonuclear cells, Bourgeois quotes Rulot and Marchal's statement that these cells are able to dissolve fibrin and that there is an extra- and intracellular action on lipase. He believes that no analogous facts have been noted in milk. We have shown that the polymorphonuclear cells commonly ingest mucinous material, and also that the macrophage cells are sometimes gorged with fat globules and assist in getting rid of red corpuscles. Bourgeois defines colostrum as a "réliquat de phagocytose d'un lait antérieurement produit, ainsi que l'ont défini—C. Porcher et L. Panisset, offre ces particularités au suprême degré." Bourgeois concludes by quoting Porcher, with whom he agrees, that "la mamelle saine est l'exception alors qu'au contraire la mamelle infectée est presque la règle".

Hopkirk (6) outlines his method of cream examination as a practical way of diagnosing mastitis. "Its general idea is the grouping of cows in a herd for milking purposes on the basis of the number of leucocytes present in the milk, as disclosed by careful microscopical examination, carried out at monthly intervals". Samples of the "fore" milk are drawn, or these may be composite samples of the whole milking. Cream smears are made with a 2-mm. loop, from an area 1.0 to 1.2 cm. in diameter. The smears are fixed lightly with heat, put through xylol and alcohol, and stained with methylene blue.

It is evident that Hopkirk's ideas and our own are similar regarding leucocytes as indicators of disease, but they differ in method of application. Our object has been to separate the cells in the milk from the cream, without injuring them or disturbing their arrangement, so that the various types of cells in association with the infective organisms may be differentiated. We have been unable to obtain uniformity in our preparations when much fat is present, or when xylol is used to remove it. Methylene blue is not a satisfactory stain, any more than it would be for a blood smear. A quick-acting blood stain, such as Hastings', gives preparations in which the various types of leucocytes may be identified. Concerning differential counts, we agree with Hopkirk that for general purposes of grouping cows in the herds, it is sufficient to indicate the approximate numbers of leucocytes. But with experience, much more than that can be determined, such as the differences that can be detected microscopically between the diseases and other abnormal conditions affecting the udder.

Methods Used in the Preparation and Examination of Samples

Before the milk sample was taken, the teats were washed with sodium hypochlorite solution, separate cloths being used for each cow, and the first few streams were discarded. The milk was cooled with ice and examined as soon as possible. The method of making smears is compared with the Breed (3) method in the following section. In staining the smears with Hastings' stain, we have found it advisable to overstain rather than understain. If the stain does precipitate a little it can easily be removed by allowing a few drops of alcohol to run over the slides. Deep staining is necessary to show mucinous material within the polymorphonuclear leucocytes. The coloration of the smears varies considerably between normal and pathological milk. Some of the smears tend to wash off when the stain is removed. In such cases it has been found best to dry the films without washing. After drying, the excess stain is removed with alcohol.

In classifying and enumerating the leucocytes, each smear was crossed once, and when the far edge was reached it was followed around to the starting point. In this way both the middle and edge of the film were examined. This is advisable because, in the process of drying, the leucocytes tend to congregate in little pools and the larger cells run to the edges. When it was desired to make sure that no polymorphonuclears were present, a search was made along the edges. It has been noted repeatedly that cells filled with cocci tend to gravitate to the edges. The large epithelial scales apparently do not follow this rule and may be found anywhere on the smear. In films so thickly covered with leucocytes that they touch one another, these distributions are not as noticeable.

Plate cultures of the milk samples were made in the usual manner with blood agar plates. In order to obtain unbiased results, the cultural and microscopic examinations were conducted separately. Stained smears were diagnosed by one author (S. H.), and later these results were compared with the cultural data obtained by the other author (R. G.).

A COMPARISON BETWEEN THE BREED AND SEDIMENT METHODS OF COUNTING LEUCOCYTES IN MILK

In the Breed method (3), 10 cc. of whole milk is shaken and 0.01 cc. spread over 1 sq. cm. on a slide. Alcohol and xylol are used to fix the smear and to remove the fat. The smears are made in duplicate. A methylene blue stain is used.

In the sediment method, 10 cc. of milk is centrifuged for 10 min. at 2500 to 3000 r.p.m. The cream is wiped out of the neck of the sample bottles with cotton. The sediment removed by a platinum loop holding about 0.005 cc. is spread over a surface of 2 sq. cm. on a slide. The smears are air-dried and stained with Hastings' blood stain. The field covered by the microscope is 0.16 mm., as advocated by Breed.

The results of a comparison between these two methods on the milk of eight cows are given in Table I. The figures show a similarity between the sediment and Breed counts in the low numbers, but otherwise are generally much higher by the former method.

TABLE I
COMPARISON OF SEDIMENT AND BREED COUNTS

Cow	Quarter	Leucocytes per 100 fields		Blood agar plate cultures
		Sediment	Breed	
1	RF	9	9	Clean
	RH	6	6	2 colonies bacilli
	LF	24	19	Diphtheroids
	LH	9	4	1 diphtheroid
2	RF	20	18	1 colony cocci
	RH	15	17	Clean
	LF	3	17	2 colonies
	LH	7	26	Diphtheroids numerous
3	RF	26	11	3 colonies bacilli
	RH	45	10	Diphtheroids
	LF	44	9	Diphtheroids
	LH	38	13	Diphtheroids
4 (Newly freshened)	RF	17	15	13 colonies coarse cocci
	RH	29	39	10 large cocci
	LF	27	15	5 large cocci
	LH	37	13	4 large cocci
5	RF	22	*12	1 colony diphtheroids
	RH	23	11	Diphtheroids
	LF	2610	372	36 colonies staphylococci, 2 diph.
	LH	29	91	No colonies
6	RF	40	28	1 colony
	RH	5	6	1 colony bacilli
	LF	2330	394	5 colonies staphylococci
	LH	29	26	5 colonies bacilli
7 (Newly freshened)	RF	109	13	1 colony contaminants
	RH	171	19	No colonies
	LF	455	136	46 colonies staphylococci
	LH	13	16	A few diphtheroids
8 (Newly freshened)	RF	88	102	9 colonies cocci
	RH	21	9	8 colonies large cocci
	LF	1130	57	30 colonies staphylococci
	LH	15	10	No colonies

Cows 1, 2, and 3 show infection with diphtheroids. Prior to the last test they had shown no organisms and very few leucocytes.

Except in special instances, such as the above and the attempt to define normal milk, we have not made complete leucocyte counts, but simply estimates of the numbers. In pathological milk it is often difficult to distinguish many of the leucocytes sufficiently to make a reliable count, although in

numerous samples the cells stain as perfectly as they do in blood. Pathological samples in which the leucocytes are scarce occur fairly often in mastitis. This scarcity is owing to a lack of resistance on the part of the cows, which would be passed as clean if the leucocyte count was relied on alone. With the Breed method it was impossible to identify as high a percentage of cells as with ours, because of inferior staining. Breed counts alone do not furnish a definite diagnosis for mastitis, but merely indicate the number of leucocytes present.

The principal reason for adopting the sediment method is because it is a simple and direct way of examining milk. If the sediment samples are taken carefully and examined quickly, the only organisms present are those that have come directly from the udder. The arrangement of the leucocytes may be informative. For example, clumping indicates a defensive reaction; numerous lining cells indicate a destructive infection, and the causative organisms will probably be seen; phagocytic activity of the leucocytes may indicate whether a case of mastitis is in the acute or chronic phase. Other abnormalities in milk may be seen, such as blood, mucin, calcium calculi, and various micro-organisms. The concentration of the leucocytes in the sediment is sufficient in most instances to give a clear picture of the reactions that are taking place.

An Attempt to Establish the Normal in Cows' Milk

Munch-Petersen (11), in a complete survey of the literature on bovine mastitis, draws the following conclusion: "What constitutes normality so far as the bovine udder and mastitis are concerned? Nowhere in the literature perused in preparing this summary has the writer found adequate work on this fundamental important point." "The question whether the udder is normally sterile or is inhabited by micro-organisms — — — cannot be said to have been definitely settled."

In view of the divergence of opinion among various workers, it is desirable to state as clearly as possible what degree of cleanliness and freedom from infection has been sought in this attempt to define normal milk. Regardless of the implication, and disregarding the previous figures we have published, it was decided to re-examine the smears from the cleanest herd that we have been testing regularly, which consists of 60 cows. From each quarter, 10-cc. samples were drawn and smeared as indicated above.

The following standards were applied to the samples, which were examined without any knowledge of their origin:

The milk must be free (i) from all visible forms of micro-organisms, whether they be considered harmless or not; (ii) from leucocytes and fixed tissue cells, except those that may be cast off in the normal process of milk secretion; (iii) from polymorphonuclear leucocytes or other cells that occur during infection; (iv) from pathogenic organisms on cultural examination.

RESULTS FOR COWS MEETING STANDARDS FOR NORMALITY

As a result of the examination of milk from the 60 cows, it was found that nine were giving normal milk. The differential cell counts are given in Table II. The figures represent the total number of each type of cell found in samples from the four quarters of each cow. Large and small forms of mononuclear cells are counted together. Many of the smaller ones showed little cytoplasm, and there were few of the large cells. The fat-bearing and epithelial lining cells are considered identical (Figs. 23, 24, 27). Epithelial scales come from the teat canal (Figs. 20, 21). Blue bodies are small, round, nuclear remains derived from the mononuclear cells (Figs. 19, 22). They stain a dark blue. Similar bodies, staining a paler blue, are believed to be globules of mucin. They are sometimes quite large, and are especially numerous in colostrum. Broken-down nuclear remains lacking cell walls were not counted.

TABLE II
DIFFERENTIAL CELL COUNTS IN MILK FROM NORMAL COWS

Cow No.	Age, years	Number of cells						No. of fields counted
		Mono-nuclear	Fat-bearing and lining	Red	Epithelial scales	Blue bodies	Poly-nuclear	
1	3	42	28	0	0	Many	0	218
2	3	46	32	0	1	Numerous	0	211
3	3	26	25	1	2	Fairly numerous	0	226
4	2½	27	14	0	0	A few	1	241
5	2½	27	15	3	1	A few	0	222
6	3	19	15	2	Large strip	A few	0	197
7	5	5	7	15	1	None	0	260
8	3½	18	8	44	3	A few	0	226
9	3	21	15	5	4	A few	1?	238

A re-examination of cows No. 1, 2, 3, and 4 was made nine months later, and all four animals were found to be clean, as formerly, although No. 3 showed four polymorphonuclear leucocytes on a patch of mucinous material. One cow that showed a few polynuclears on the first test became clean on the second; another was clean on the first test, but showed diphtheroids and 17 polynuclears on the second. It is significant that when the data on the nine clean cows were compiled in Table II, it was found that all these animals were young.

Having defined normality in one herd, we attempted to find other normal animals by examination of two small herds, and three more cows have been added to the list.

DISCUSSION

Although the personal equation must be considered in a histological method, the principal requirement in our standard of normal cow's milk is the absence of polymorphonuclear leucocytes, and these cells are unmistakable in their active form. This standard is difficult to attain. Therefore, freedom from

polymorphonuclears in milk is a very good sign. In support of our view, we quote from Maximow and Bloom (8), who state that in the lactating mammary gland in man "granular leucocytes are rare, and the presence of a noticeable number of them is always an indication of abnormal inflammatory changes, which have induced the migration of these elements from the blood vessels".

There are two rather puzzling conditions in cow's milk which should be mentioned here because of their bearing on normality. The first is the common occurrence of red blood corpuscles, accompanied by a certain number of leucocytes that are connected with the normal process of repair. The cause of haemorrhage into the udder is discussed later. The second condition is more difficult to explain. The mucinous state of the milk seen in newly calved cows may call forth large numbers of polymorphonuclear leucocytes, which will be found gorged with the mucin; later both mucin and leucocytes disappear. We have seen this condition described as normal. In our experience there is a variation in the amount of mucinous material produced, as only one or two quarters of the udder may be involved. This condition may disappear entirely. The same may be said of non-pathogenic organisms.

It would appear that normality in milk, as we have defined it, may exist at one period and not at another, which is what must be expected. Finally, it should be stated that in our attempt to define normality we have chosen a standard that would classify a very large percentage of cows as abnormal, judging by our results. We have not used such a drastic standard in grouping clean and diseased cattle in all our herds.

RESULTS FOR COWS NOT MEETING STANDARDS FOR NORMALITY

The standard for normality was not met by 51 cows of the herd of 60. Nine gave milk free from diphtheroids and other pathogenic organisms on culture, except for one occurrence of *S. aureus*; but leucocytes were numerous in five of these and polymorphonuclear cells were found in the others.

Diphtheroid Carriers

The remaining 42 cows, or 70% of the herd, showed epithelial scales infected with *Corynebacterium bovis*. Of these, 28 cows were infected with diphtheroids only, and 14 with diphtheroids associated with other organisms.

Twenty-eight cows were infected with diphtheroids not associated with any other organisms that could be detected by microscope or by culture. Summarized in age groups, these showed the following results:— the 9 oldest cows (6 to 13 years), few polymorphonuclear cells in 7, fairly numerous in 2; eight or more diphtheroid-bearing cells in 4, average of three in 5 (compare Table III): 6 cows (5 years), fairly numerous polymorphonuclears in 4, a few in 2; average of three diphtheroid-bearing scales in 5, more than eight in 1: 4 cows (3 to 4 years) few polymorphonuclears in 3, fairly numerous in 1; three diphtheroid-bearing scales in 3, several in 1: remaining 9 as below.

For comparison with the nine normal cows of Table II, in which cultures of milk samples were negative for pathogenic organisms, the findings for nine

young diphtheroid carriers are given in Table III. It can be seen that polymorphonuclear cells are more numerous in the latter group, although a few cows showed such small numbers that they would almost have appeared to be clean but for the infected epithelial scales. In Nos. 17 and 33 it was difficult to form a correct estimate, owing to the numbers of degenerate leucocytes present.

The polymorphonuclear counts are classified according to location of the cells in the smears, to show the larger numbers on the edge of the film. The epithelial scales were located with a low-power lens and then examined for diphtheroids with the oil immersion. It is probable that by following this method very few are overlooked.

TABLE III
POLYMORPHONUCLEAR CELLS IN MILK FROM COWS INFECTED WITH DIPHTHEROIDS

Cow No.	Age, years	Polymorphonuclear cells		Epithelial scales with diphtheroids
		Within smear	Edge of smear	
13	4	9	14	8
14	4	2	3	4
15	4	4	18	2
16	4	1	7	4
17	4	21	75	Several
25	3	1	18	1
26	4	4	8	6
31	2½	2	3	Several
33	2½	23 pyknotic	53 around scales	Numerous

Approximately 200 fields counted for each cow.

It appears that the older cows react less to diphtheroids than the younger. In this connection, attention might be drawn to cows Nos. 1, 4, and 5 (Table II), which became infected with diphtheroids since 1937, and in 1938 showed numbers of polymorphonuclear leucocytes. This occurred in the absence of infective organisms other than diphtheroids.

Fourteen cows were infected with diphtheroids associated with other organisms. Three of these had streptococcic mastitis. Seven showed large numbers of leucocytes associated with cocci. One showed cocci with few leucocytes. One had many leucocytes with contaminating organisms. Two had many leucocytes without organisms being found on culture.

In our herd of 60 cows 70% were infected, a much higher percentage than the 24% infected in 300 cows examined in the ordinary way. Most workers incline to the view that diphtheroids are harmless organisms. Martinaglia (7) stated that he had not found any evidence of leucocytic clumping around the bacilli. Hopkirk (6) suggests that micrococci and diphtheroids are not as toxic as some organisms and puts them in the same category.

We have encountered clumps of leucocytes surrounding groups of diphtheroids, and also polymorphonuclear cells ingesting the bacilli. Our evidence

points to an increase of polymorphonuclear leucocytes caused by the presence of diphtheroids (Figs. 16, 17, 21). But as regards pathological lesions, we have not found anything that points to an injurious effect on the udder. The diphtheroid-bearing scales are desquamated from the teat canal. The bacilli stain a deep purple with Hastings' and lie mainly on the surface of the scales. The scales themselves have an orange shade.

Bacteriological Examination of Diphtheroid Strains

Twenty-five strains of diphtheroids were submitted to bacteriological examination. These were isolated from the milk of cows in a number of herds. They were seeded in litmus milk and in beef extract broth containing Andrade's indicator and lactose, maltose, dextrose, dextrin, glycerol, mannitol, saccharose, salicin, arabinose, raffinose, and inulin. No fermentation had occurred in any of these tubes after 11 days of incubation at 37° C. Litmus milk was slightly darker in colour after two weeks of incubation, and a marked grey sediment appeared.

Two recently isolated strains were seeded in sterilized milk. Intraperitoneal injection of 2 cc. of 4-day milk culture had no effect on two guinea pigs. The growth from a 48-hr. blood-agar slant injected intraperitoneally into two other guinea pigs was also innocuous. Subcutaneous injection of the same amount caused a slight dermal thickening after a couple of days and then disappeared.

These strains coincide with the description of *Corynebacterium bovis* sp. nov. (1).

Staphylococcic Mastitis

The characteristic appearance of staphylococcic infections in milk under the microscope is of a multitude of polymorphonuclear leucocytes, many of which are ring-shaped (Fig. 1). They are evidently filled with a colourless material, which has pushed the nucleus over to the periphery of the cell. We have been unable to determine what this material is. The large number of these gorged-looking cells offers a very different picture to that of streptococcic mastitis. Staphylococci are not difficult to detect, as a rule; they may be found within clumps of leucocytes (Fig. 1). When phagocytosis is active they do not appear in large numbers within the cells (Fig. 3), while in some cases the leucocytes are able to control them completely and the cultures are sterile. Sometimes the polymorphonuclears will become so filled with cocci that they reach the bursting point (Fig. 15). These overloaded cells tend to gravitate to the edge of the film. We have not seen the macrophages ingesting either the cocci or the polymorphonuclear leucocytes in milk. Cocci are found in the lining cells from the ducts, and when this condition is observed it shows conclusively that the cocci are damaging the udder (Fig. 4). The cocci are very numerous in some samples, forming large clumps that spread out on all sides and almost obliterate the leucocytes. When the milk becomes "stringy", which is mainly due to shredding of the polymor-

phonuclear leucocytes, the cocci tend to disappear and the smear becomes almost free from cells except for a few of the mononuclear elements, which lie in the spaces between the broken-down cells. A few days later the fibrinous material disappears and there is a return to a more natural secretion from the udder. Unfortunately, the cocci are likely to reappear, accompanied by leucocytes.

During the course of the disease there are times when the leucocytes are few and the cocci numerous, lying free between the cells or crowding around them without being phagocytized. This would indicate a negative phase or lack of resistance on the part of the host. Staphylococci have been noted on the surface of epithelial scales from the teat canal. In colostrum the polymorphonuclears are active in picking up mucinous material. It is believed that they perform a useful function in getting rid of mucin, and also in phagocytizing cocci that may be present in the new milk. In any event, the leucocytes, mucin, and cocci disappear shortly. Mucinous material is not as commonly seen in staphylococcal infections as in streptococcal, while macrophage cells generally are equally numerous.

In view of the previous discussions on leucocyte counts, Table IV is given to show the changes in the leucocyte count in one animal (Case 2 of Table V). In the milk samples taken on June 25th, leucocytes were not numerous in the right quarters. In the left quarters, the leucocytes and cocci were five times more numerous. On August 26th, all quarters showed very large numbers of leucocytes and in three no cocci were seen microscopically. In the fourth quarter a few cocci were noted, and only a few colonies were found on culture.

TABLE IV
VARIATION IN NUMBERS OF ORGANISMS FOUND IN A CASE OF STAPHYLOCOCCIC MASTITIS
(Case 2)

Date	Quarter of udder	Polymorphonuclears, %	Mononuclears		Lining cells, %	Fields counted	Total no. leucocytes counted	Staphylococci
			Small, %	Large, %				
June 25	RF	70	11	2	17	100	180	Free
	RH	66	13	2	19	100	217	Free and in leucocytes
	LF	57	23	4.5	15.5	20	160	In leucocytes
	LH	55.5	21	2.5	21	20	180	Free and in leucocytes
Aug. 26	RF	39.5	36.5	6.5	17.5	2	400	None
	RH	41.5	32	6	20.5	2	600	None
	LF	26	48	12.5	13.5	2	440	None
	LH	70.5	10.5	3	16	2	460	A few

Typical protocols recording the occurrence of leucocytes and cocci in three cases of *Staphylococcus aureus* infection are given in Table V, which shows how the disease may assume a chronic state. Case 1 became infected with *Streptococcus mastitidis* at the end of the period of observation reported here.

In Case 1 the staphylococci were missed once by microscopic examination when culture indicated they were present. In Case 2 the cocci were missed twice, and in Case 3, once. In the latter, both microscope and culture failed to show the organisms on Aug. 26.

TABLE V
EXAMINATION OF MILK FROM COWS WITH STAPHYLOCOCCIC MASTITIS

Case No.	Date	Leucocytes	No. affected quarters	Staphylococci found by	
				Microscope	Culture
1	Sept. 17, 1935	Numerous	3	In polys.	+
	Dec. 19	A few	4	In polys.	+
	Mar. 24, 1936	Many	4	Free and in polys.	+
	May 7	Many	3	Free and in polys.	+
	26	Many	3	Free and in polys.	+
	June 5	Many	4	Free and in polys.	+
	11	Many	4	None	+
	18	Many	1	In polys.	+
	25	Many	2	In polys.	+
	July 2	Many	3	Free	+
	9	A few	0	Free	+
	Aug. 26	Many	2	In polys.	+
	Jan. 11, 1937	Many	2	Free and in polys.	+
	April 13	Many	3	Free and in polys.	+
	July 13	Fairly numerous	2	In 1 quarter, also strep.	+
	Oct. 18	Many	1	Free	Strep. +
					Strep.
2	Dec. 19, 1935	None	0	—	—
	Mar. 26, 1936	A few	4	In polys.	+
	May 7	Many, fibrinous material	2	—	+
	12	Many	1	In polys.	+
	June 5	Many	1	—	+
	11	Many	2	Free	+
	18	Many	1	In polys.	+
	25	A few	4	In polys.	+
	July 2	Many	1	In polys.	+
	9	Many	2	In polys.	+
	Aug. 26	Many	4	A few in polys.	A few
3	Mar. 24, 1936	Fairly numerous	1	In epithelium	+
	May 7	Many	2	In polys.	+
	12	A few	1	Free and in polys., many	+
	June 5	Fairly numerous	1	—	+
	11	Fairly numerous	2	In polys.	+
	18	Many	3	In polys.	+
	25	Many	1	In polys.	+
	July 2	Many	3	In polys.	+
	9	Many	2	In polys.	+
	Aug. 26	A few	0	—	—
	Oct. 15	A few	0	In polys.	+

Streptococcic Mastitis

A typical case of streptococcic mastitis, in which there are long chains of streptococci, is easily recognized microscopically (Fig. 8). These cases, however, are not very common. Others occur in which a few chains of cocci

are visible, or in which they are almost impossible to find. The appearance of a typical smear is different from that in other forms of mastitis, because the mononuclear cells (Fig. 2) are generally more numerous and among them are large macrophage cells filled with fat globules (Fig. 18). The total number of leucocytes is less than it is in staphylococcal infection. Mucinous material is commonly seen, even in cases of long standing. It is often ingested by the polymorphonuclears and stains a bright blue colour (Fig. 29). Lining cells are numerous, indicating injury to the udder. Clumping of the cells is usual; these form loose, irregular clumps, the polymorphonuclears being in separate groups with small and large mononuclears and macrophages around them. Blood corpuscles may be present also.

The mononuclears and macrophages show a different form of degeneration to the polymorphonuclears. The nucleus becomes fragmented, leaving round bodies of various sizes in the cell (Fig. 19). This has been seen so often that it seems worthy of note. In the acute stages there occurs an almost complete destruction of the polymorphonuclears, which shred to form fibrinous strands. In this process many small round bodies of chromatin are liberated and scattered over the smear.

The above microscopic picture suggests streptococcal infection even before the chains of cocci have been found. When these are rare, they may be seen in the polymorphonuclear cells. A difficulty arises here, for when cocci are visible in a cell one must make sure that there is definite chain formation (Fig. 13). Fortunately, there is often a prolongation of the chain outside the cell. The chains may also be seen on the surface of epithelial scales (Figs. 9, 20). It would appear that these scales afford neutral ground upon which organisms may develop, as we have repeatedly found streptococci and staphylococci growing upon them; especially is this true of diphtheroids, which occur there almost exclusively. Streptococci may also be found on desquamated lining cells.

Another indication that the case is streptococcal is the absence of other forms of organisms. In doubtful cases we have found Bryan's (4) method of incubating the sediment useful. It is not a bad plan to examine the four smears on each slide quickly at first, with a high-power dry lens. If streptococci are

TABLE VI

VARIATION IN NUMBERS OF ORGANISMS FOUND IN A CASE OF STREPTOCOCCAL MASTITIS

Quarter of udder	Polymorphonuclears, %	Mononuclears		Lining cells, %	Fields counted	Total no. leucocytes counted	Staphylococci
		Small, %	Large, %				
1	35	29	13	23	5	200	Free and in polys.
2	36	40	7	15	70	100	None
3	58	21	10	11	78	100	Numerous, free
4	64.5	18.5	10	7	10	330	Rare, in polys.

detected in one of the quarters, the slide can then be more carefully examined. Even the presence of abnormal numbers of leucocytes places a cow in the doubtful category, and failure to find streptococci in such a case merely means that the test should be repeated.

Table VI is appended to show the wide variation in the numbers of streptococci and to show the different reactions taking place simultaneously in the udder of an animal. The leucocytes were numerous in two quarters, 1 and 4; they were held in a mucinous network in quarter 4, and mucin was seen in the polymorphonuclears (Fig. 29).

Typical protocols recording the occurrence of leucocytes and cocci in three cases of *Streptococcus mastitidis* infection are given in Table VII.

TABLE VII
EXAMINATION OF MILK FROM COWS WITH STREPTOCOCCIC MASTITIS

Case No.	Date	Leucocytes	No. affected quarters	Streptococci found by	
				Microscope	Culture
1	Dec. 19, 1935	—	—	—	—
	27	—	—	—	—
	Mar. 24, 1936	Many	2	In polys.	+
	May 7	Many	3	In polys.*	+
	July 9	A few	—	None	—
	Aug. 26	A few	—	Free	—
	Jan. 11, 1937	A few	—	In polys.*	—
	April 13	Many	1	None	+
2	July 13	A few	—	In polys.	+
	Dec. 19, 1935	—	—	None	—
	Mar. 24, 1936	Many	3	In polys.*	+
	May 7	Many	2	In polys.*	+
	26	Many	1	In polys.*	+
	July 9	Many	1	In polys.*	+
	Aug. 26	Many	3	2 chains	+
	Jan. 11, 1937	A few	—	None	+
	April 15	Many	2	In polys.*	+
	July 13	Many	2	Free and in polys.	+
3	Oct. 11, 1935	—	—	—	—
	Dec. 19	—	—	—	—
	Mar. 24, 1936	—	—	—	—
	May 7	Many	1	Staph.	+
	12	Many	1	Free	+
	July 9	Many, fibrinous	2	Short chains	+
	Oct. 15	A few	—	—	—
	Jan. 11, 1937	Many	1	—	+
	April 13	A few	—	—	+
	July 13	Many	2	Short chains	+

* Doubtful whether streptococci or staphylococci.

† Non-pathogenic forms of cocci.

B. coli Mastitis

Milk samples from 10 cases of *B. coli* mastitis have been examined. In four cases the most outstanding abnormality was the large number of lining cells from the udder (Fig. 7). In one of these, it looked as though a considerable

part of the lining had been sloughed out; in the other three, the lining cells were numerous. Red blood corpuscles were also noted. The polymorphonuclear leucocytes were degenerating and shredding in six cases. Bacilli were common in eight, chromatin granules were numerous in three cases (Fig. 6). Mucin was noticeable in two cases, bacilli being very numerous in the mucinous patches.

Corynebacterium pyogenes Mastitis

Seven lots of samples from cows infected with *Corynebacterium pyogenes* have been examined. The most prominent feature under the microscope was the large number of organisms seen; among them occasional coccoid forms occurred. The polymorphonuclears phagocyte many of the organisms, then break down and liberate them all over the field. These broken-down polymorphonuclears gather in large tangled skeins, and chromatin granules are noticeable. Lining cells and crenated red blood cells are numerous.

Phagocytosis and Destruction of Cocci

In a discussion of resistance to diseases of the udder, it is well to consider it briefly as indicated by the effect of the invading organisms on the cells of the udder, and by their effect on the milk. The organisms that grow and multiply in the udder damage the cells of the alveoli and ducts, as seen by the presence in the milk of degenerate lining cells associated with these organisms. There is reason for believing that the damage is partly, perhaps mainly, superficial. The leucocytes that are attracted to the affected part migrate into the lumen of the ducts and attack the organisms. The latter may be living on the surface of the tissues or in the descending stream of milk, where they are in an ideal medium for growth. The attempt of the leucocytes to attack these organisms, with subsequent loss to the animal of both, must be responsible for a very heavy drain of leucocytes from the blood. Yet effective resistance to infection in the udder is indicated clinically, because one frequently sees only one or two quarters of the udder affected at one time. With these general observations in mind, the microscopic observations will be discussed.

In support of the view that the polymorphonuclear cells produce antibacterial substances, we have repeatedly noticed in contaminated milk samples that the contaminants grew feebly or not at all in the quarters in which many leucocytes occurred. The milk from leucocyte-free quarters of the same animal would be heavily contaminated.

When large numbers of leucocytes are present in milk, it often means that few or no micro-organisms will be found microscopically or culturally. This has been observed in both staphylococcic and streptococcic infections. It is followed by resolution, when the leucocytes disintegrate and disappear. On other occasions numerous leucocytes and organisms have been seen degenerating simultaneously. The process of resolution, as observed in milk, differs from that described for the serous cavities, where the macrophages

phagocyte the damaged polymorphonuclear leucocytes. In milk we have not observed the macrophages phagocytizing the polymorphonuclear cells, but they do pick up the small mononuclears (Fig. 28), often in large numbers. They also ingest mucin, red corpuscles, and nuclear remains.

In staphylococcic mastitis, infected leucocytes are not as numerous as one would expect, except in those cases in which there is low resistance and the leucocytes are few in number. Commonly, small clumps of five to six polymorphonuclears will be encountered with a few cocci scattered outside them. At other times large, gorged cells will be seen quite replete with cocci.

In streptococcic mastitis the evidences of resistance are easier to detect than they are in staphylococcic, because when chain formation becomes irregular it is readily seen. In several samples the cocci appeared to be granular wherever the chains touched or lay across a polymorphonuclear cell (Fig. 11), and normal where they did not touch. From these observations it appeared that the lytic agent was inside the cells rather than in the surrounding fluids. In other instances the streptococci were among clumps of polymorphonuclears showing signs of degeneration (Fig. 14), the chains were short, and the cocci granular and irregular in size, suggesting an extracellular effect upon them (Figs. 10, 12). Atypical forms have been noticed repeatedly. This would suggest that they are meeting opposition in their growth and may have lost a certain amount of virulence. We have seen little evidence of encapsulation in our samples.

In conclusion, certain general statements may be made regarding the microscopic examination of milk sediment. When resistance is high there will be many leucocytes and few organisms present. When many organisms and few leucocytes are found, it is probable that a new invasion of the udder will take place. Active phagocytosis indicates a return to normality.

Leucocytic Reactions Occurring in Non-Infected Swellings of the Udder

Injuries to the udder occasionally occur that are followed by resolution without infection, as indicated in the following brief record. Cultures of all milk samples were negative.

Record of Heifer

When this heifer calved there was a hard swelling in the LH quarter. Blood clots were milked out. Cow first examined about three weeks later. The LH quarter was fairly hard but not painful.

Day 1. RF, RH, and LF quarters clean. LH quarter: Many monos, polys, macros, clumping. Red blood corpuscles numerous.

Day 7. RF, RH, and LF quarters clean. LH quarter: Numerous monos and lining cells in clumps. Not many polys. Macrophages picking up red cells, which had become less numerous. No organisms seen.

Day 14. RF, RH, and LF quarters: A few polys, monos, and lining cells. LH quarter: Fairly numerous monos, lining cells, and macros. Polys

more numerous on islands of mucin which they were ingesting. No organisms seen. Swelling reducing.

Day 22. RF, RH, and LF quarters clean. LH quarter: Fairly numerous monos, lining cells, and small aggregations of polys. No organisms seen. Observations ceased.

We have recently encountered another case that was similar to the above. This serves to show that in cows such cases do occur, though they are probably not common.

Red Corpuscles in Milk

Red blood cells are of common occurrence in milk, and they are often unaccompanied by any signs of infection. The enormous distension of the udder that often occurs probably causes the blood vessels to rupture; in samples of colostrum we have seen much blood, which supports this explanation. The red corpuscles usually appear well shaped and normal. In severe infections, like *B. coli* or *C. pyogenes*, the appearance of the red cells is different; they are crenated and often stain badly (Fig. 15). In a few samples the milk has had a dark red coloration with few corpuscles visible under the microscope, which is caused by rupture of the cells and liberation of haemoglobin. Macrophage cells picking up the corpuscles have been repeatedly observed. It would appear that when milk is bloody it is frequently caused by mechanical injury to the udder, and in a smaller number of cases it is connected with disease.

Chromatin Staining Granules and Shredding of Leucocytes

Chromatin granules may occur in large numbers, sometimes covering the entire slide (Fig. 6); fibrinous strings are generally associated with them. They represent a breaking-down of the polymorphonuclear leucocytes and also of the lining cells from the milk ducts. Both these degenerative products are found when there is complete destruction of the cells. In the early stages of the process, formation of the chromatin granules is quite noticeable before the cells break down (Fig. 5). These changes have been noted frequently in our records.

Muir (10), writing about the destruction of cocci in man, says "the polymorphonuclear leucocytes are seen to be in a degenerated condition, their nuclei being fragmented and changed into deeply staining chromatin globules". It is evident, therefore, that the changes in milk closely resemble those that occur in the tissues in other parts of the body.

Calcium Phosphate Deposits in the Teats and Udder Tissue

According to several authorities on pathology, calcium is deposited in the tissues as a result of some form of injury. In the udder, bacterial infections are common and mechanical injuries also occur frequently. The parts involved are soft, secreting tissues, which are not intended to be handled roughly. Possibly a combination of infection and mechanical injury may cause the deposits in the walls of the teats, but in the udder it is more likely that infection

is responsible, because large numbers of calculi have been found quite high in the outer portions of the walls (Fig. 25). According to Muir (10), dead cells and old collections of pus may become the starting point of calcification. Fatty tissues also undergo calcification in some cases. In an indurated teat a complete ring of calcium was found just below the milk cistern. Dr. H. M. LeGard informs us that in his practice he has frequently been called on to remove small calculi from the teat canal. Sweet, Miller and Graves (12) record that many lobules showed numerous small concretions or milk calculi in the alveoli, *post-mortem* in the udders of five cows. They did not comment on the origin or significance of these calculi in the udder.

The recent Australian Milk Commission Report (11) does not list any reference to calcium deposits in milk nor to the calculi in the tissues.

In autopsies on dairy cattle at the Toronto abattoirs a number of calculi were detected, and in one case about a gram of small calculi was picked out of the tissues. These calculi were tested by H. W. Lemon of this Foundation, who reported they were probably a mixture of di-calcium and mono-calcium phosphate.

DETECTION OF CALCULI IN MILK

It was found much simpler to detect calculi in the tissues than in the milk sediment. Even now, though a considerable number of cases have been recorded, it is often difficult to determine the nature of certain bodies encountered on the slides. Microscopically, the calculi show concentric rings and appear dark in colour with crenated edges, closely resembling those found in the udder tissues (Fig. 26).

When calculi are plentiful in a milk smear they may alter the aspect of the stained preparation, giving it a more open, granular appearance, and when the finger is passed over the surface it gives the sensation of sand paper. Calculi have been repeatedly observed in certain of our cows, sometimes accompanying disease, at other times in healthy animals. Further examinations will have to be made before anything more definite can be said about the frequency of their occurrence in milk.

Colostrum

Colostrum being the first milk secreted by glands that have previously been non-functional, it was decided to examine the udder in pregnant heifers. Sections were cut, giving information as to the type of cells that would be found in the new milk. In a young heifer, pregnant about three to four months, it was found that the two hind teats were patent and milk was being secreted in these quarters. The front teats were still closed. The alveoli showed secreting and non-secreting portions. In the secreting tissues the alveoli were circular and the walls thicker, the lining cells and nuclei rounder,

than in the non-secreting areas. These findings would support the statement of Porcher and Panisset, quoted by Bourgeois (2), that colostrum is composed of the remains of milk previously secreted.

In colostrum there is much debris and large numbers of nuclear remains, which we have called blue bodies in our records (Fig. 22). These are sometimes accompanied by a multitude of mucin-like globules. The figures of colostrum bodies given by Maximow and Bloom (8) bear a resemblance to Fig. 23 in this paper, which represents the nuclei of lining cells from the ducts accompanied by strands of fat globules. These nuclei are commonly encountered. A pale-blue-staining nucleolus can usually be detected within the nucleus, but the cytoplasm that surrounds the cells in the alveoli has disappeared in the colostrum. A few small mononuclear cells and numbers of macrophages with fat droplets occur in some specimens. Polymorphonuclear cells have been noted, actively phagocytosing the mucinous globules, in the absence of micro-organisms. Red blood corpuscles are numerous. They are irregularly distributed, being very plentiful in one quarter and scarce in another. They mean little as far as the identification of colostrum is concerned.

We have examined some 20 samples of colostrum. The smears are generally coloured a dark blue with Hastings' stain, owing to the mucinous material present. When leucocytes are numerous the colour changes to red. Apparent infections that rapidly cleared up have been found in several cases, and we have subsequently passed some of these cows as clean. It is generally stated that colostrum only persists a few days, though in a few of our samples we have been able to detect nuclear remains and mucinous material for more than a fortnight.

Acknowledgments

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PLATE I

FIG. 1. *Staphylococcic* infection. Note ring-shaped polymorphonuclears. FIG. 2. Leucocytes in streptococcic mastitis. The mononuclear cells predominate. In such cases the milk may be microscopically and culturally sterile. FIG. 3. Polymorphonuclear leucocyte containing two pairs of cocci. FIG. 4. Lining cells from the milk ducts infected with staphylococci. FIG. 5. Degenerating leucocyte containing two rows of chromatin globules. FIG. 6. Chromatin globules scattered over field. These are derived from the breakdown of the polymorphonuclear cells. FIG. 7. *B. coli* mastitis. Note numbers of lining cells.

PLATE II

FIG. 8. Actively growing chains of streptococci from milk sediment. FIG. 9. Streptococci growing on an epithelial scale, with a ring of polymorphonuclears surrounding them. FIG. 10. Atypical chains of cocci growing free in milk. No leucocytes are attacking them. The club-shaped ends and enlarged grains suggest that the medium is not favourable. FIG. 11. The chain of cocci that crosses the central cell shows degenerative changes where it touches the cell. Beyond the cell, the cocci appear normal. Degenerate cocci are also visible in the upper cell. FIG. 12. The streptococci are being destroyed in the lower cell, and above them a few small chains appear normal. FIG. 13. Single polymorphonuclear cell with short chains of streptococci. In samples where cocci are scarce, the finding of one or two infected cells is a great help in diagnosis. FIG. 14. Streptococci being actively phagocyted by the polymorphonuclears. FIG. 15. The large central polymorphonuclear cell is gorged with micrococci. Red blood corpuscles are numerous and the leucocytes are degenerating.

PLATE III

FIG. 16. Epithelial scales from the teat canal bearing diphtheroids, some of which are free, others being phagocyted around the edges. FIG. 17. Clump of polymorphonuclears phagocytting diphtheroids. FIG. 18. Macrophage cells that have ingested fat droplets. These fat-bearing cells are common in streptococcic mastitis, but occur in lesser numbers in other forms of mastitis. FIG. 19. Nucleus of leucocyte breaking into round masses that occur in large numbers in colostrum. FIG. 20. Epithelial scale bearing diphtheroids and chains of streptococci. FIG. 21. Characteristic appearance of diphtheroid-bearing scale. FIG. 22. Colostrum. Nuclear remains (called blue bodies in the text).

PLATE IV

FIG. 23. Nuclei of lining cells from udder with fat droplets. They have lost their cytoplasm. These cells are numerous in various affections of the udder and in colostrum. FIG. 24. Lining cell surrounded by a ring of fat globules which are attracted to the cell. FIG. 25. Calcium concretions in section from udder. Note crenated borders. FIG. 26. Calcium deposit in milk sediment. Note similarity in shape to Fig. 25. FIG. 27. Normal lining cells that have broken away intact from the walls of the udder. FIG. 28. Macrophage cell filled with small mononuclear cells. FIG. 29. Polymorphonuclear cells ingesting mucinous material.

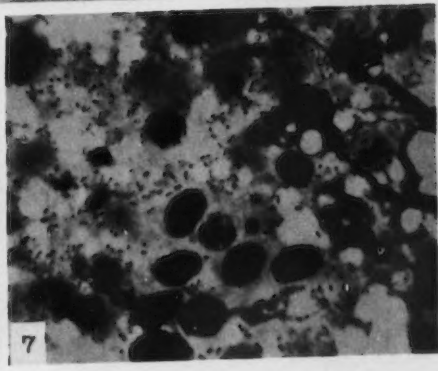
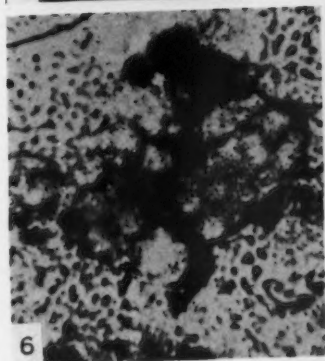
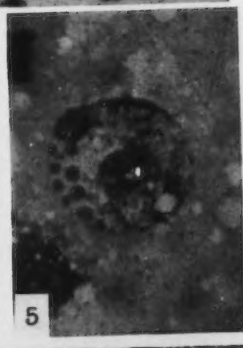
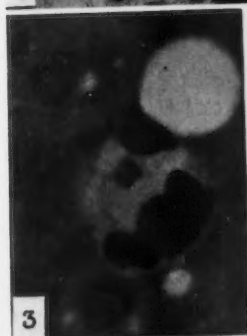
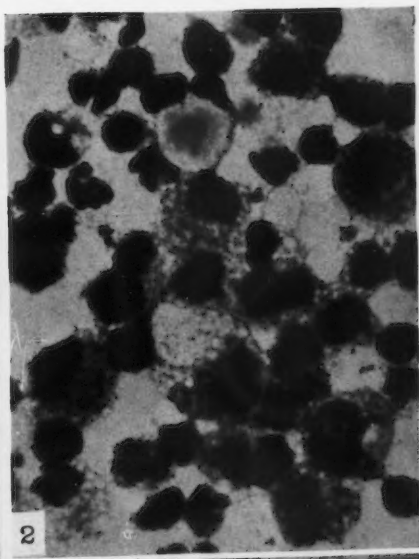
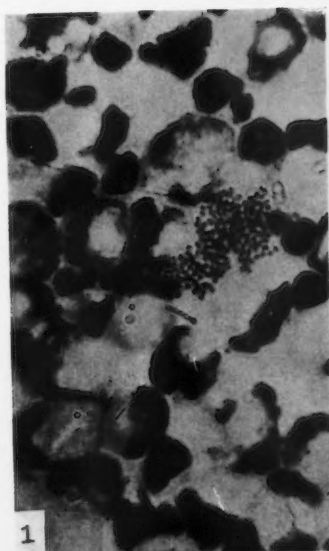
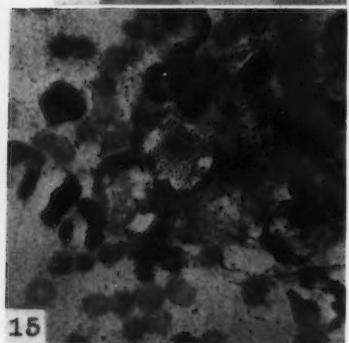
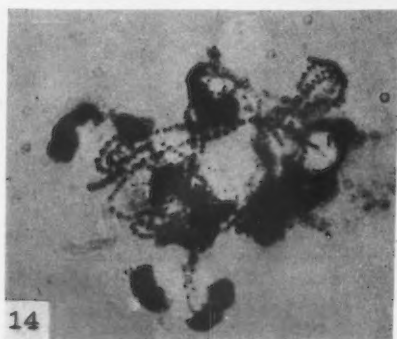
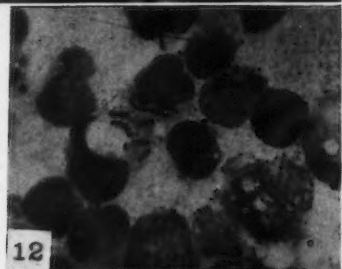
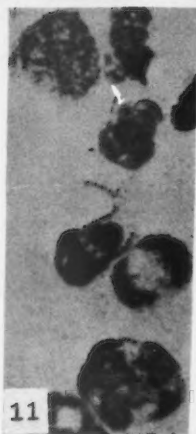
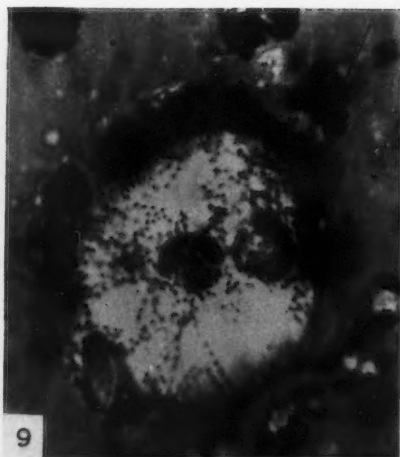
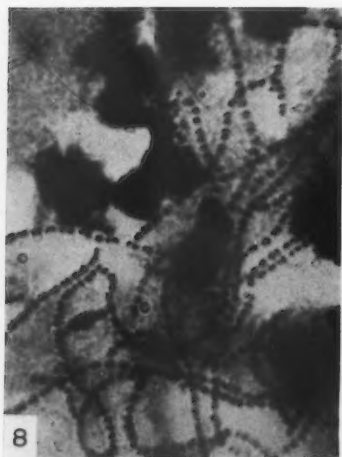


PLATE II



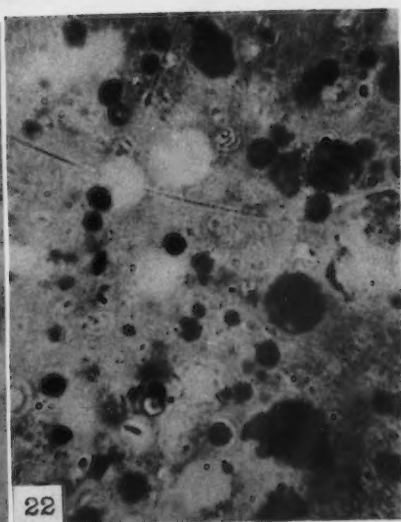
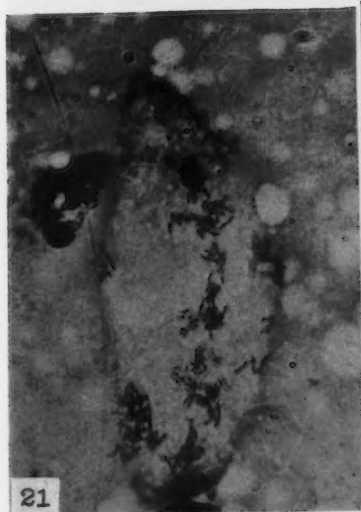
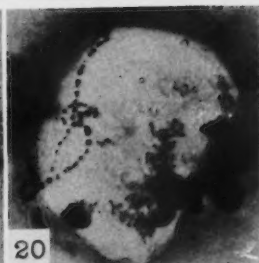
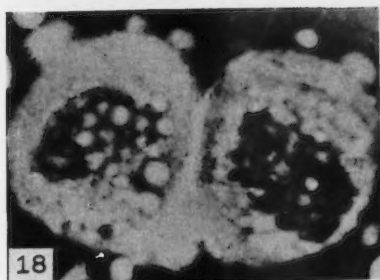
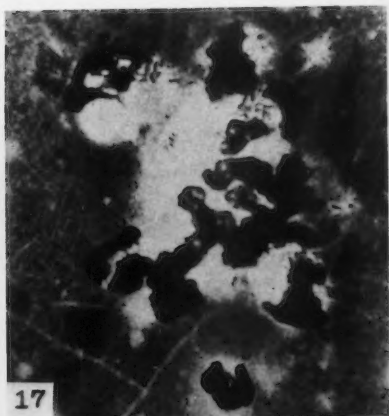
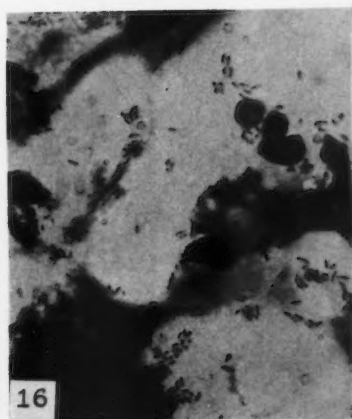


PLATE IV

